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ANATOMY OF SUGARCANE STALK AS INFLUENCED BY TOP BORER (*SCIRPOPHAGA NIVELLA* F.) ATTACK

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Received May 26, 1947

I. Introduction

The reaction of the tissues of sugarcane stem to mechanical injury sustained as a result of an attack by top borer (*Scirpophaga nivella* F.) and their subsequent structural variations, so far as the authors are aware, have not been reported anywhere. Pemberton (1925) and Stewart and Hansson (1928) reported injury to roots by centipedes and nematodes and there are passing references by Ferret (1927) and Lyon (1927), about the formation of galls in sugarcane stem, the internal structure of which has not been paid the attention it deserves. Morbid anatomy induced by various pathogens and insect pests explains the behaviour of the host to a great extent.

II. Material and Methods

Four to five shoots attacked by top borer of Co 205, Co 210, Co 213, Co 299, Co 313 and Co 331 were collected before the secondary invasion by other organisms took place. The affected portion of the stalk was cut into internodes at various stages of growth. Hand sections were stained with Safranin and mounted in Canada balsam, in the usual way.

III. Observations

The examination of slides showed that the reaction of sugarcane tissues to the attack of top borer was confined to the neighbourhood of the actual damage, and the maturer the tissue, the less the area involved; and so also, the less the deviation from normal structure. Both the parenchymatous matrix and vascular bundles were more or less equally affected. The derangement of the tissues manifested itself mainly by the changes in size and shape of cells and the staining quality of their walls.

In very young top-most internodes, two or three layers of parenchymatous cells adjacent to the tunnel usually grew into long palisade-like tissue (Plate I, Fig. 1) deep into the cavity which became irregular in outline, some times their distal ends forming knobs. The outgrowths brought about by the rapid increase in the size of newly formed cells, in the absence of proper tissue tension all round them, narrowed the borer tunnel to a mere slit in some internodes. Safranin stained the walls of the morbid

cells much more deeply than those of the healthy ones. In partially mature internodes, where the growth rate had slowed down, these cells could not acquire their normal size though there was not much difference between a healthy and an affected cell as regards their shape (Plate I, Fig. 2). In very young internodes also this type of reaction by parenchymatous tissue situated between the palisade-like and healthy tissue was sometimes met with. The staining quality of the cell wall also changed as stated above. In fully mature internodes, the reaction to the attack and the presence of a larva was confined to about half a dozen layers of cells all round the cavity. The cell walls became thick and highly lignified (Plate I, Fig. 3) with the result that the healthy tissue was cut off from the cavity and its contents. A prototype of this lignified ring between the healthy tissue and the morbid growth was often met with in younger internodes also. Sometimes the cells outside the sanitary cordon, lost their rounded shape and became polygonal (Plate I, Fig. 4) thus obliterating the intercellular spaces, which appeared to be a further attempt at the segregation of healthy tissues. Excepting the radial elongation of a few cells isolated or in groups, there was no change in the shape or size of the parenchymatous cells surrounding the borer tunnel in fully developed internodes (Plate I, Fig. 4).

Disruption in the case of vascular bundles was equally extensive. There were no vascular bundles in the deranged parenchymatous matrix, due to the complete disorganisation of the primordia of vascular bundles (Plate I, Figs. 1 and 2). Their frequency and structure slowly moved towards the normal as one receded from the cavity. Elongation of xylem end of the vascular sheaths towards the tunnel, the suppression of protoxylem vessels, and distortion of one or both of the metaxylem vessels or their complete absence were among the most common deformities (Plate I, Fig. 5). Phloem was not so easily affected as xylem. Sometimes its place was found to have been occupied by thick-walled cells probably intruders from the adjoining vascular sheath. The sclerenchymatous cells forming the sheath which were so characteristically polygonal in outline when healthy, became diamond-shaped in cross-section with their longer axis towards the cavity. Their walls could not acquire the usual thickness and were comparatively poorly lignified. Vascular bundles far removed from the centre of disturbance in the growing internodes were found to be quite normal in all respects (Plate I, Fig. 5). But in mature internodes even those abutting the cavity did not show any structural change (Plate I, Figs. 3 and 4). Very often their xylem components, less so the phloem, were found to have been choked with a dark, darkish brown or chocolate coloured substance which was insoluble in water, various grades of alcohol and xylol. Sometimes this

substance filled a few of the parenchymatous cells adjacent to the cavity. But in the case of the vascular bundles, it was found to be present at considerable distance from the cavity (Plate I, Fig. 6).

No marked difference in the behaviour of tissues of the varieties studied, was noticeable except that the choking of vascular bundles appeared to be more common in Co 210 than in others.

IV. Summary

1. The stage of growth at which the tissues were attacked determined the affected area and the changes in the size and shape of cells forming them.

2. Lignification of cell walls was greater and less respectively in affected parenchyma and sclerenchyma than in the healthy ones.

3. No marked varietal differences in the behaviour of tissues were discernible.

V. Acknowledgements

The work was carried out as part of the Sugarcane Research Scheme in Bihar being financed jointly by the Bihar Government and the Indian Central Sugarcane Committee to whom grateful thanks are due.

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Explanation of Plate

Microphotographs of Transverse Sections showing Reactions of Stem Tissues at Various Stages of Plant Growth

(Magnification × 50 for all)

Fig. 1. Young internode showing abnormally elongated cells of the parenchymatous matrix without any vascular bundle.

Fig. 2. Partially mature internode. The tunnel is surrounded by a few layers of parenchymatous cells smaller than the healthy ones. (*a*) a partially developed vascular bundle with protoxylem suppressed, metaxylem not fully formed, and poorly lignified vascular sheath.

Fig. 3. Mature internode, showing a highly lignified ring of cells cutting off the tunnel from healthy tissue.

Fig. 4. Mature internode. The parenchymatous cells become polygonal. A few of those abutting the cavity become elongated; vascular bundles normal.

Fig. 5. Immature internode showing common deformities of vascular bundles, such as elongation of xylem-end of vascular sheath, suppression of protoxylem and distortion or suppression of metaxylem.

Fig. 6. Mature internode. Vascular bundles normal but choked with a blackish substance,

RESPIRATION OF SUGARCANE IN RELATION TO ESSENTIAL PLANT NUTRIENTS

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Received May 28, 1947

I. Introduction

Mitscherlich and colleagues (quoted from Willcox, 1937) have shown that from the point of view of nutrient requirements, nitrogen element is the one that is required in maximum quantities followed by potash, phosphorus and other elements such as calcium, magnesium, sodium, silicon, iron, etc. Other workers have studied the effect of deficiency of essential elements on the respiratory activity of plant. Thus, Gregory and Richards (1929) and later on Richards (1932) studied the effect of manurial deficiency and observed that the curve of response exhibited a fall soon after the element became deficient. Similarly Lyon (1926, 1927) studied the effect of phosphatic deficiency on plant respiration. Hammer (1936) described the effect of nitrogen supply on the rate of metabolism in plants and showed a marked increase in the respiration rate on the application of nitrates to tomato and wheat plants. Arnon (1937) reported an increased rate of respiration even in the case of excised barley roots on the application of nitrates. Warbung and Negelani (Arnon, 1937) showed that the respiratory quotient of *Chlorella* increased following the absorption of nitrates. Nightingale, *et al.* (1931) observed the effect of calcium deficiency on nitrate absorption and on metabolism in tomato plants. Studies by Parija and Saran (1934) indicated that starved leaves exhibited an enhanced rate of respiration in a deficient environment.

Suzaki and Kenjo (1935, 1936, 1937), Kenjo (1938) and Saito and Kenjo (1939) published the results of their experiments on the effect of deficiency of nitrogen and phosphorus on leaf colour, growth of stalks and leaves, development of roots, sucrose content of juice, total ash content, etc. These experiments were performed in water cultures but the effect of nitrogen and phosphorus deficiency on respiration rate was not determined.

In the studies reported hereunder, an attempt was made to study the metabolic rate of excised shoots of plants raised in sand cultures deficient

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in nitrogen, potash and phosphorus and in the absence and presence of minor elements. Dry weight of plants was determined at the close of the experimental period to elucidate the interrelationship among the type of nutrient medium and respiratory process and growth of plants. And, therefrom, it was proposed to indicate the degree to which the various elements act as limiting factors in plant metabolism.

II. Material and Methods

The experiments were performed in glass jars measuring 24″ × 18″ × 10″. These were filled with quartz sand that had been washed repeatedly to get rid of all extraneous matter. Equal weighed quantities of sand were put in each of the glass jars, enough of water being added to saturate the sand to its full capacity. Three one-eyed setts obtained from central three nodes of fully developed cane stalks were planted on 3–11–38 at 2½″ depth in each of the glass jars. The setts were 2″ in length and all weighed approximately equal, the range in the weights being 31·12 and 27·08 grammes. The germination of setts was duly recorded day after day. Most of the setts germinated within a week of the germination of the first sett in the experiment, but another week was allowed before the rest of the ungerminated setts were taken out. The pots were then so arranged (Table I) that the distribution of plants might as far as possible be equal with regard to date of germination.

Table I

Showing the arrangement of pots for various nutrient treatments (Nov., 1938)

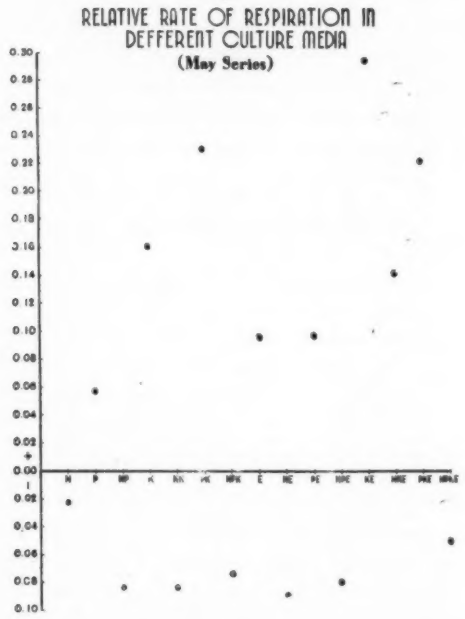
Sl. No.	Treatment	Reference	Pot No.	Date of germination			Pot No.	Date of germination			Pot No.	Date of germination		
				1	2	3		1	2	3		1	2	3
1	Control ..	A	26	18th	16th	16th	21	14th	18th	23rd	28	18th	..	..
2	N ..	B	13	21st	16th	15th	9	19th	20th	16th	35	16th	21st	25th
3	P ..	C	1	14th	13th	12th	2	19th	19th	15th	30	14th	23rd	25th
4	N P ..	D	7	14th	15th	14th	24	14th	..	..	16	23rd	20th	..
5	K ..	E	38	13th	16th	12th	41	25th	18th	17th	19	..	23rd	20th
6	N K ..	F	3	13th	17th	13th	32	19th	19th	19th	17	19th	20th	..
7	P K ..	G	4	14th	16th	14th	40	19th	26th	15th	44	20th	23rd	20th
8	N P K ..	H	10	20th	20th	16th	39	20th	..	..	46	18th	17th	17th
9	E	I	6	21st	19th	14th	14	..	21st	18th	37	14th	..	..
10	N E	J	15	16th	21st	22nd	25	..	..	17th	43	18th	17th	15th
11	P E	K	8	16th	14th	18th	31	..	17th	..	45	..	17th	17th
12	N P E	L	11	21st	21st	17th	42	19th	17th	14th	47	14th	25th	26th
13	K E	M	5	16th	14th	19th	27	15th	21st	19th	29	..	..	26th
14	N K E	N	18	15th	14th	18th	33	18th	19th	20th	48	18th	21st	..
15	P K E	O	12	13th	14th	14th	23	17th	21st	21st	36	14th	..	..
16	N P K E	P	20	21st	19th	17th	22	17th	19th	15th	34	23rd	..	17th

Remarks.—E Treatment refers to combination of salts when salts other than N, P_2O_5 or K_2O were supplied.

After the rearrangement of pots the solutions containing the various nutrient salts were added. The culture media used was modified by Knop's solution as recommended by Shull and Loomis (1938), and balanced for sand culture work as suggested by McCall (1916). Various salts were replaced according to the scheme given by Shull and Loomis (*loc. cit.*) for excluding N, P_2O_5 or K_2O as the case may be from the full culture solution. But where only N, P_2O_5 or K_2O were to be applied separately or in combination, as shown in Table I, the same salts as used for the culture solution, were employed. For the control treatment, distilled water was used every time the solution was applied in the pots.

The plants were allowed to grow till the beginning of May when respiration studies on A and C plants were taken up. Excised shoots constituting the leaf tuft bearing two leaves below the standard (transverse mark) leaf

Fig. 1



were used for the investigation. Method of gaseous exchange was employed for conducting respiration experiments. The apparatus described by Luthra and Cheema (1931) was used for the purpose with the slight modification as given by Khanna and Raheja (1938). This modification consisted in the use of a tower filled with pumice-stone saturated with concentrated

sulphuric acid to free the air of all moisture before entering the respiration chamber. From the CO_2 evolved, respiration rates were worked out on the basis of per gram fresh weight of material.

The one plant left over was allowed to grow till about middle of November. The respiration rates of excised shoots were worked out as above for each of the single plants in the pots. Almost simultaneously with the above determination, the respiration of whole plants were also determined. Besides measurements of length and maximum width of the last three fully matured leaves were also recorded. Further notes were taken on the leaf colour, nature of the leaf tuft and extent of the leaf tip drying.

III. Experimental Results

The respiration data obtained during the month of May and November are presented below (Table II), while Fig. 1 in the text shows the relative rate of respiration of plants during May in the various cultural media (Table II).

The plotted points are the mean deviations from the control obtained by summing up all the values that are not asterisked in Table II minus the mean value for control and have been given (Table III, Column 11). It will be noticed that the respiratory activity of plants grown in culture media containing nitrogen salts except in the treatment N.K.E. is low (negative). On the other hand, the application of potassium salts increased, in general, the rate of respiration. P_2O_5 alone or in combination with K_2O increased the metabolic activity. Effect of E alone increased the respiration rate (Table III).

It will, however, be observed that the number of observations (Column 3, Table III) for each varied, and there were considerable variations in the respective values of the respiration rate of plants grown in culture media. To obviate the difficulty of unequal numbers, for working out the approximate analysis of variance, ' Effect Values ' were derived by the method described in the appendix of the paper. These values have been graphed in Fig. 2.

It is observed that effect of nitrogen, potassium and E in the derived ' Effect Values ' was also the same. Nitrogen in every case tended to decrease the rate of metabolism of the plant. The maximum effect of increasing the rate of respiration was indicated by potassium followed by E and least that of phosphorus.

The analysis of variance as worked out for the May series is given below (Table IV).

TABLE II

Showing respiration of plants during May and November

Sl. No.	Treatment	Pot No.	Rate of respiration in c.c. per hour per gram fresh weight		
			May A	May C	November B
1	Control ..	26	0·340	0·274	1·043
		21	0·390*	0·308*	..
		28	..	..	0·871*
		13	0·184	0·254	0·354
		9	0·169	0·178	0·941
2	N ..	35	0·331	0·589	1·024
		1	0·287	0·541	0·836*
		2	0·360	0·289	0·652
3	P ..	30	0·377	0·327	0·490
		7	0·200	0·127	0·608
		24	0·311	0·253	0·389
4	N. P. ..	16	..	..	0·836
		38	0·174	0·284	0·601
		41	1·011	0·404	1·383
5	K ..	19	..	0·449*	0·534*
		3	0·285	0·177	1·285
		32	0·223	0·210	0·831
6	N. K. ..	17	1·02*	..	0·680*
		4	0·651	0·153	1·430
		40	0·386	0·430	0·366
7	P. K. ..	44	1·342	0·269	0·384
		40	0·224	0·315	0·576
		10	0·247	0·141	0·322
8	N. P. K. ..	39	..	..	..
		37	0·367	0·629	1·121
		6	0·275	0·346	0·668
9	E ..	14	..	0·548*	2·680*
		43	0·200	0·319	1·656
		15	0·166	0·202	0·742
10	N. E. ..	25	..	..	0·387*
		8	0·181	0·628	0·904
		45	..	0·114*	1·800*
11	P. E. ..	31	..	..	0·721*
		42	0·314	0·324	0·331
		11	0·204*	..	1·150*
12	N. P. E. ..	47	0·146	0·220	0·252
		5	0·374	1·438	1·349
		27	0·233	0·361	1·165

TABLE II—(*Contd.*)

Sl. No.	Treatment	Pot No.	Rate of respiration in c.c. per hour per gram fresh weight		
			May A	May C	November B
13	K. E. ..	29	..	..	1·283*
		18	0·499	0·275	0·761
		33	0·805	0·221	0·792
14	N. K. E. ..	48	0·280*	..	0·522*
		12	0·423	0·542	1·660
		23	0·706	0·448	0·728
15	P. K. E. ..	36	..	..	0·788*
		22	0·176*	0·133*	..
		20	0·359	0·144	1·724
16	N. P. K. E. ..	34	..	0·195*	0·983*

* These figures were treated as abnormal and were, therefore, not used for interpretation of the data. It will be noticed that they relate to the data obtained with plants which were either one or two in a pot, *i.e.* all three plants were not there or else in the November series, the plant had dried and therefore the respiration data was omitted.

TABLE III

Showing rate of respiration of plants in the May series

Sl. No.	Treatments	No. of observations	Rate of respiration in c.c.						Mean value	Deviations from the control values	Derived effect values
			No. 1	No. 2	No. 3	No. 4	No. 5	No. 6			
1	2	3	4	5	6	7	8	9	10	11	12
1	Control ..	2	0·340	0·274	..	..	..	..	0·307	..	..
2	N. ..	6	0·184	0·254	0·167	0·178	0·331	0·589	0·284	−0·023	−1·506
3	P. ..	6	0·287	0·360	0·377	0·541	0·289	0·327	0·364	+0·057	−0·190
4	N. P. ..	4	0·200	0·127	0·311	0·253	..	..	0·223	−0·084	−0·304
5	K. ..	4	0·174	0·284	1·011	0·404	..	..	0·468	+0·161	+0·862
6	N. K. ..	4	0·285	0·177	0·223	0·210	..	..	0·224	−0·083	−0·456
7	P. K. ..	6	0·651	0·386	1·342	0·153	0·430	0·269	0·539	+0·232	−0·192
8	N. P. K. ..	4	0·226	0·315	0·247	0·141	..	..	0·232	−0·075	−0·074
9	E. ..	4	0·367	0·629	0·275	0·346	..	..	0·404	+0·097	+0·450
10	N E. ..	4	0·200	0·319	0·166	0·202	..	..	0·222	−0·085	−0·076
11	P. E. ..	2	0·181	0·628	..	..	..	..	0·405	+0·098	−0·340
12	N. P. E. ..	4	0·314	0·224	0·146	0·220	..	..	0·226	−0·081	+0·058
13	K. E. ..	4	0·374	1·438	0·233	0·361	..	..	0·602	+0·295	+0·292
14	N. K. E. ..	4	0·499	0·275	0·805	0·221	..	..	0·450	+0·143	+0·318
15	P. K. E. ..	4	0·423	0·542	0·706	0·48	..	..	0·530	+0·223	−0·358
16	N. P. K. E.	2	0·359	0·144	..	..	..	..	0·252	−0·055	−0·184

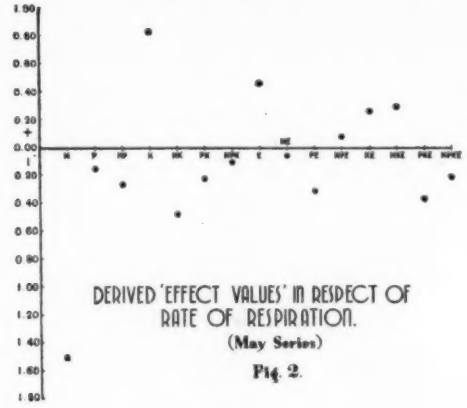


Fig. 2.

TABLE IV

Showing approximate analysis of variance of May series

Due to	D. F.	S. S.	M. S.	'F' values			
				Observed	Theoretical		
					P–0·20	P–0·05	P–0·01
N ..	1	0·141752	0·141752	1·799	1·70	4·08	7·31
P ..	1	0·002256	0·002256	0·029	do	do	do
K ..	1	0·046443	0·046443	0·590	do	do	do
E ..	1	0·012656	0·012656	0·161	do	do	do
N. P. ..	1	0·005776	0·005776	0·073	do	do	do
N. K. ..	1	0·012996	0·012996	0·164	do	do	do
N. E. ..	1	0·000361	0·000361	0·005	do	do	do
P. K. ..	1	0·002304	0·002304	0·029	do	do	do
P. E. ..	1	0·007225	0·007225	0·092	do	do	do
K. E. ..	1	0·005329	0·005329	0·068	do	do	do
N. P. K. ..	1	0·000342	0·000342	0·004	do	do	do
N. P. E. ..	1	0·000210	0·000210	0·003	do	do	do
N. K. E. ..	1	0·006323	0·006323	0·080	do	do	do
P. K. E. ..	1	0·008010	0·008010	0·102	do	do	do
N. P. K. E. ..	1	0·002116	0·002116	0·027	do	do	do
Error ..	48	3·781415	0·078779	..	..	..	..
Total ..	63	4·035514	..	..	..	..	..

From this analysis, it is apparent that nitrogen effect referred to above was the highest though not significant. Since the variations in the respiration rate were very wide, the significance values were tested at 20% level of significance but no effect came out as significant. Wherever nitrogenous salt combined with any other salt or a combination of salts, the effect was to equalise the respiration rate to that of the control series. These interesting resultshave been discussed later in the text,

The supply of nutrients was continued upto the middle of July and later the plants were starved of their respective nutrient supply with a view to obtain wide differences in the rate of metabolism in various series (Parija and Saran, *loc. cit.*). The results of the respiratory studies conducted in November are given (Table V) in the text. It will be noticed that the number of plants taken up for investigation were different for the different series. Some of these plants had completely died out while others had to be discarded on grounds similar to those reported under the former studies. Fig. 3 illustrates the mean deviations in the respiratory activity from the

Fig. 3.

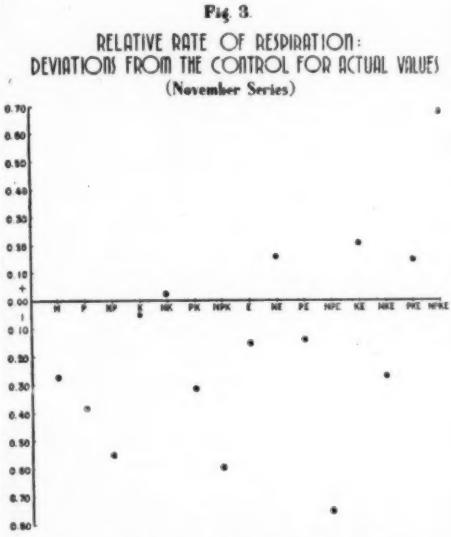
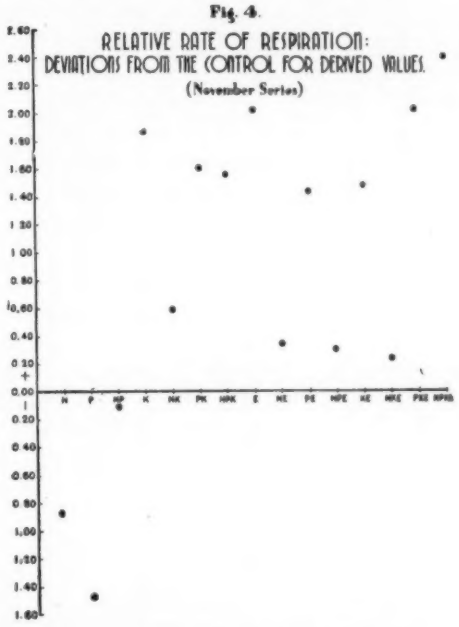


Fig. 4.



control series (Table V, Column 8). The respiratory activity was low when either nitrogen or phosphorus was applied and it was high when potash was given to the plants. The results were not found to exactly correspond with those stated above. Derived values (Fig. 4) were, therefore, worked out by the same method as given in the Appendix.

It is obvious that wherever potassium salt was supplied in nutrient culture media, the rate of katabolic process was high. Application of nitrogen in the form of calcium nitrate [$Ca(NO_3)_2$], however, tended to decrease the rate of respiration except in the combinations N P K and N P K E. Effect of E (combination of minor elements) was to enhance the rate of respi-

TABLE V

Showing the rate of respiration of plants in the November series

Sl. No.	Treatments	No. of observations	Rate of respiration in c.c.			Mean value	Daviations from the control	Derived effect values
			1	2	3			
1	2	3	4	5	6	7	8	9
1	Control ..	1	1·043	..	..	1·043	..	14·439
2	N ..	3	0·354	0·941	1·024	0·773	−0·270	−0·901
3	P ..	3	0·836	0·652	0·490	0·659	−0·384	−1·545
4	N. P. ..	2	0·608	0·389	..	0·498	−0·545	−0·141
5	K. ..	2	0·601	1·383	..	0·992	−0·051	+1·915
6	N. K. ..	2	1·285	0·831	..	1·058	+0·015	+0·575
7	P. K. ..	3	1·430	0·366	0·384	0·727	−0·316	+1·567
8	N. P. K. ..	2	0·576	0·322	..	0·449	−0·594	+1·475
9	E ..	2	1·121	0·668	..	0·894	−0·149	+2·041
10	N. E. ..	2	1·656	0·742	..	1·199	+0·156	+0·385
11	P. E. ..	1	0·904	..	..	0·904	−0·139	+1·521
12	N. P. E. ..	2	0·331	..	0·252	0·292	−0·751	+0·329
13	K E. ..	2	1·349	1·165	..	1·257	+0·214	+1·409
14	N. K. E. ..	2	0·761	0·792	..	0·776	−0·267	+0·137
15	P. K. E. ..	2	1·660	0·728	..	1·194	+0·151	+1·997
16	N.P.K.E. ..	1	1·724	..	..	1·724	+0·681	+2·381

TABLE VI

Showing approximate analysis of variance of November series

Due to	D. F.	S. S.	M. S.	'F' values			
				Observed	Theoretical		
					P−0·20	P−0·05	P−0·01
N ..	1	0·050738	0·050638	0·217	1·79	4·49	8·53
P ..	1	0·149189	0·149189	0·638	do	do	do
K ..	1	0·229201	0·229201	0·980	do	do	do
E ..	1	0·260355	0·260355	1·114	do	do	do
N. P. ..	1	0·001242	0·001242	0·005	do	do	do
N. K. ..	1	0·020664	0·020664	0·088	do	do	do
N. E. ..	1	0·009264	0·009264	0·039	do	do	do
P. K. ..	1	0·153468	0·153468	0·656	do	do	do
P. E. ..	1	0·144590	0·144590	0·618	do	do	do
K. E. ..	1	0·124080	0·124080	0·531	do	do	do
N. P. K. ..	1	0·135977	0·135977	0·581	do	do	do
N. P. E. ..	1	0·006765	0·006765	0·029	do	do	do
N. K. E. ..	1	0·001173	0·001173	0·005	do	do	do
P. K. E. ..	1	0·249251	0·249251	1·066	do	do	do
N. P. K. E. ..	1	0·354323	0·354323	1·516	do	do	do
Error ..	16	3·740210	0·233763	..	..	..	..
Total ..	31	5·630490	..	..	..	..	..

ration. Analysis of variance (Table VI) revealed that the variations were higher for E, P K E and N P K E. The effects for P K, N P K and K E were also fairly high. Though none of the effects is significant, it will be noticed in the above combinations that both in the absence and presence of E, the effect of K, P K and N P K was more or less similar. Evidently K being common to all, its effect on respiration was throughout very marked. These differences in the effect of nutrient salts on the rate of respiration of foliage have been discussed later in the text.

B. Dry weight data.—Dry weight studies were carried out for the November series and the results (Table VII) when arranged in descending order of yield were N K E, N P K E, N P K, N P E, N P, N, N E, P E, P K E, P, K E, N K, K, P K and E.

TABLE VII

Showing dry matter percentage of plants for the November series

Sl. No.	Treatments	Dry matter of plants (gm.)				Mean	Deviations from the control
		No. 1	No. 2	No. 3	Total		
1	Control ..	12·04	13·96*	23·60*	12·04	12·04	..
2	N ..	26·64	27·64	27·00	81·28	27·09	+15·05
3	P ..	13·79*	19·33	15·20	34·53	17·26	+ 5·22
4	N. P. ..	30·13	29·04	64·06*	64·17	32·09	+20·05
5	K ..	10·51	12·89	11·30*	23·40	11·70	− 0·34
6	N. K. ..	13·54	20·87*	14·64	28·18	14·09	+ 2·05
7	P. K. ..	12·96	10·24	10·62	33·82	11·27	− 0·77
8	N. P. K. ..	43·74	39·17	52·67*	82·91	41·46	+29·42
9	E ..	7·74	11·80*	10·19	17·93	8·97	− 3·07
10	N. E. ..	27·03	81·93*	23·94	50·97	25·49	+13·45
11	P. E. ..	19·58	31·61*	17·78*	19·58	19·58	+ 7·54
12	N. P. E. ..	36·90*	39·63	34·78	74·41	37·21	+25·17
13	K. E. ..	16·04	15·47	21·24*	31·51	15·76	+ 3·72
14	N. K. E. ..	44·61	45·13	55·58*	89·74	44·87	+32·83
15	P. K. E. ..	18·05	16·58	30·64*	34·63	17·32	+ 5·28
16	N. P. K. E. ..	43·69	43·99*	37·99*	43·69	43·61	+31·57

* Dry matter values for those plants of which the results of respiration were omitted have not been taken into consideration while working out the mean.

From the order stated above, it is evident that the application of nitrogen throughout was more effective than that of either phosphorus or potash in increasing the accumulation of dry matter in the plant. Except in the treatment N K E the combined effect of N P was much more than that of P or N alone. Effect of K, unless it was in combination with N P, was always to depress the yield factor. The relationship between the respiratory process and the yield factor has been discussed subsequently.

IV. Discussion and Results

According to the fourth principle of agro-biology, a certain definite amount or intensity of growth factor produces a certain definite result (Willcox, 1930). In other words, the effect is a quantitative one. In an ensemble of growth factors also, the effect of any one of them is additive or cumulative. In the experiments reported above this has been clearly brought out in both the respiratory activity of plants carried out in May and that completed in November. At both the periods, the specific effect of nitrogen was to reduce the rate of respiration while addition of K, or E, except in combination with N P, was always to enhance the rate of respiration. It appears that nitrogen by virtue of its being a rapidly absorbed anion brings about more effective equilibrium in the rate of metabolism, that is to say, the accumulation of substances with nitrogen is higher and, therefore, the carbon dioxide output per unit of accumulated substance is low. No other substance reduces the respiratory quotient of the plant like nitrogen. In May series particularly, the effect of the presence of phosphorous salts, was to increase the rate of katabolic process. Combined with nitrogenous salts it was more effective in reducing the rate of respiration. Potassium salts in conjunction with N or P showed the least effect in reducing the rate of katabolism. But when all the big three—N, P and K combined, the effect was to reduce the rate of respiration. The combined effect of the salts other than those having nitrogen, phosphorus or potash was to enhance the breakdown of the metabolisable material and this was irrespective of the big three elements. The age of the plant did not matter in this respect because the specific effect was identical in both the May and the November series.

In the case of dry matter output, nitrogen in combination with the rest was most effective; the next in order being the combination of nitrogen and phosphorus. Effect of potash alone or in separate combination with nitrogen, phosphorus, nitrogen plus E, and phosphorus plus E was rather deleterious to the accumulation of dry matter.

From the results of the respiratory activity of plants and the accumulation of assimilated products, one may infer that potash is the first limiting factor of the three big ones, for it is the earliest to show its deleterious effect under the conditions of the experiment described above. The antagonism of salt exists so long as it does not combine with nitrogen and phosphorus but this is not less apparent because of the presence of high or low concentration of growth factors. This is clear from the similarity of the results in respect of respiration obtained from May series, when plants were maintained at the normal salt requirement, and from the November series, when the plants had been kept starved.

V. Summary

Investigations on metabolic activity of plants were carried out in sand culture to study the specific influence of nitrogen, phosphorus, potash and combination of other minor elements, alone and in combination with one another. Respiration experiments were performed on plants grown upto November. The application of nutrient supply was stopped in mid-July when plants were starved with a view to study the antagonistic effect under normal and abnormal conditions of growth. The dry matter of plants harvested in November was also estimated. The results arrived at were as follows:

(i) Nitrogen decreased the rate of respiration. It was most effective in reducing the rate of respiration when it was applied in combination with all the other elements.

(ii) Effect of potash was just the opposite to that of nitrogen. It enhanced the rate of respiration unless it was present in combination with N P.

(iii) Phosphorus alone increased the rate of metabolism. But in combination with nitrogen it was very effective in reducing the rate of respiration and in combination with potash in keeping up a higher rate of respiration.

(iv) Effect of the combination of salts of the minor elements alone was to increase the rate of katabolic processes. In combination with nitrogen, phosphorus or potash, this effect was much less apparent.

(v) Nitrogen was most effective in the accumulation of dry matter; phosphorus was the next best while neither potash nor combination of other salts of minor elements effected any increase in the dry matter.

(vi) Potash has been shown as the first limiting factor as it is the earliest to show adverse effect on the respiratory process and it maintains its antagonism so long it does not combine with nitrogen and phosphorus together.

(vii) The antagonistic effect on respiratory process is maintained both under normal and abnormal conditions (starvation) of nutrient supply.

VI. Acknowledgements

The work was carried out as part of the Sugarcane Research Scheme in Bihar being financed jointly by the Bihar Government and the Indian Central Sugarcane Committee to whom grateful thanks are due. The assistance rendered by Mr. K. S. Bandyopadhyay, Statistician at the Station, in the statistical analysis of the data presented in the paper is also acknowledged.

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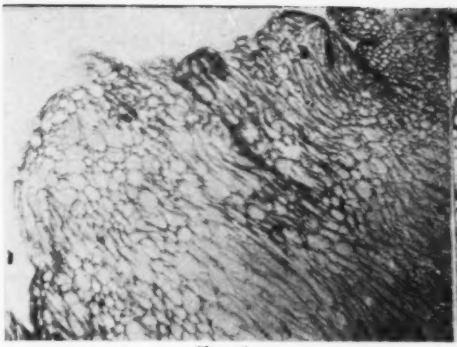

FIG. 1

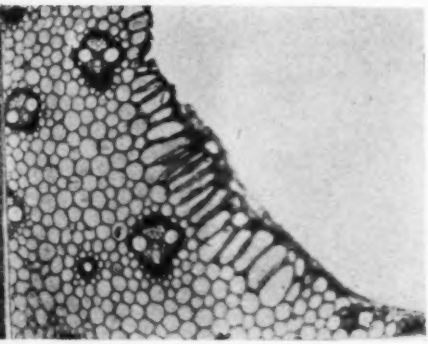

FIG. 4

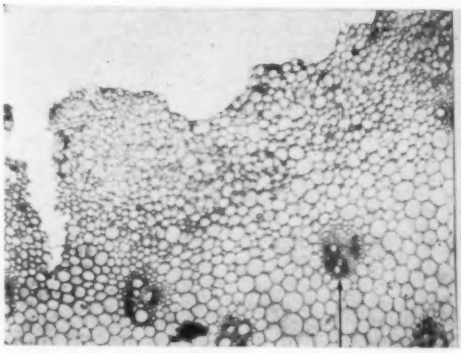

FIG. 2 *a*

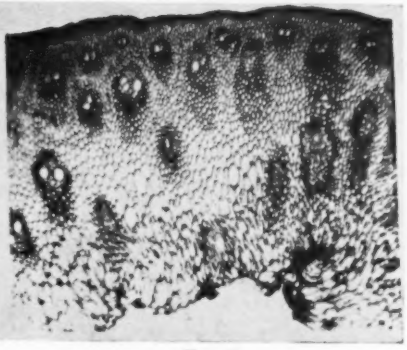

FIG. 5

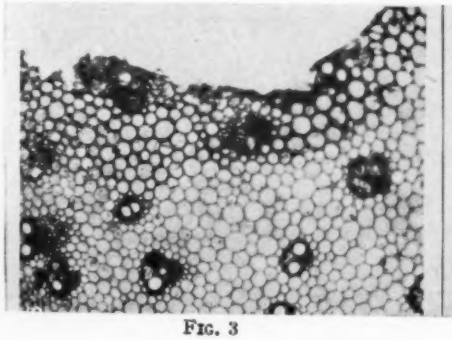

FIG. 3

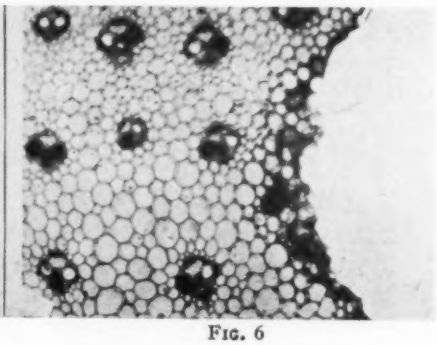

FIG. 6

APPENDIX

The experiment consists of four treatments (N, P, K and E) at two levels each, and is therefore of the type 2^4. Presence and absence of a treatment are its two levels. Denoting the presence of a treatment by the suffix 1 and its absence by zero, the main effects and interactions may be represented as follows:

$$N = \tfrac{1}{8}(N_1 - N_0)\ (P_1 + P_0)\ (K_1 + K_0)\ (E_1 + E_0)$$

$$N\,P = \tfrac{1}{8}(N_1 - N_0)\ (P_1 - P_0)\ (K_1 + K_0)\ (E_1 + E_0)$$

$$N\,P\,K = \tfrac{1}{8}(N_1 - N_0)\ (P_1 - P_0)\ (K_1 - K_0)\ (E_1 + E_0)$$

$$N\,P\,K\,E = \tfrac{1}{8}(N_1 - N_0)\ (P_1 - P_0)\ (K_1 - K_0)\ (E_1 - E_0)$$

. . . . . .

. . . . . .

and so on for the other main effects and interactions.

Each of the above expressions, which stands either for a main effect or an interaction represents 1 degree of freedom, and when expanded, will consist of 16 terms, 8 of which are positive and 8 negative and the estimate of a main effect or an interaction will be obtained by substituting the value (rate of respiration) for each term in the expression obtained after expansion.

In comparing these effects amongst themselves, the multiplying factor '$\frac{1}{8}$' has been omitted throughout and "Effect Values" have been derived by substituting for each term (*e.g.*, $N_1\,P_1\,K_0\,E_0$) in the expression, the mean value of respiration for all the pots occurring under the treatment combination ($N_1\,P_1\,K_0\,E_0$).

In getting the sum of squares due to any of these main effects or interactions in the *approximate* analysis of variance, the arithmetical value of the expression (effect value) corresponding to the relevant effect has been squared and then divided by 16. 16 has been used as the dividing factor, because each expression has been evaluated from 16 averages.

BOMOLOCHUS ACUTA N.SP., A COPEPOD PARASITIC ON THE GILLS OF *DUSSUMIERIA ACUTA*

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Received April 9, 1947
(Communicated by Prof. S. G. M. Ramanujam, M.A., Ph.D., F.A.SC.)

A SINGLE mature female specimen of this Ergasilid parasite was found attached to the gills of the Madras Rainbow Sardine *Dussumieria acuta*. A full description is given here since our knowledge of copepods parasitic on Indian fishes is very scanty as indicated in a previous paper (6) and since the Ergasilidæ which are not far removed from the free living forms consist of only a few genera and species and the earlier descriptions are far from accurate. The parasite measures 2·72 mm. long and 0·75 mm. across the cephalothorax, the widest part of the body. The cylindrical egg sacs, 1·32 mm. long, form a conspicuous feature. Behind the semicircular broad cephalothorax the rest of the thoracic segments succeed in decreasing width and end in a narrow abdomen lying between the two long egg sacs. The cephalothoracic shield or carapace is not merely convex above, concave below, but has an edge fringed by a softer thinner extension of the ventral plate, helping the adhesion of the parasite by suction. This is facilitated further by a deeper caving in of the ventral surface brought about by the folding dorsalwards and forwards of the cephalon just behind the maxillipedes and in front of the first swimming leg of the thorax. As a result, the cephalothorax serves as a large sucker bounded in front by the frontal area, on the sides by the thick edge of the carapace and behind by the first swimming legs. The cephalothorax is semicircular in outline with the straight posterior side 0·75 mm. wide, fused to the first free thoracic segment along the whole width. Anteriorly the frontal region is marked by a deep median notch to the bottom of which is attached a frontal plate. This elliptical plate is folded transversely in the middle in such a way that the front half hangs down. The anterior (*i.e.*, the lower) margin bears two processes with swollen bases and acuminate tips. The dorsal convex surface of the carapace is marked by a median furrow starting close to the hind border of the cephalothorax. As it runs forwards, the furrow widens into a valley flanked by two ridges which end in two conspicuous prominences one on either side

of the median notch containing the frontal plate. In the anterior region of the valley just behind the frontal lobe is located the median eye which recalls strongly the median eye of free living copepods. It is of three parts, two lateral and one anterior and median. In the vicinity of the eye, the carapace is marked by several denticles of varied size and form.

Appendages.—The cephalothorax bears the six appendages of the cephalon and that of the first thoracic segment fused with the head. As

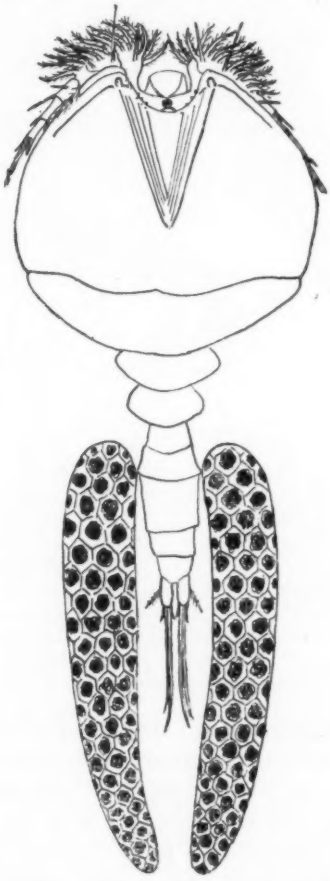

FIG. 1. *Bomolochus acuta* n. sp. Dorsal view of female × 63. (Shown without thoracic appendages).

there are many errors in the earlier descriptions of Bassett Smith, Scott, Brian and others, occasioned by defective observation and interpretation of

these appendages, the author of the present paper has adhered to Wilson's descriptions. The first antennæ are large and setose. Each consists of seven segments of which four form the proximal half and three form the distal part of the appendage. The first or basal joint widens anteriorly and bears two plumose setæ, the second joint, which is more dorsal, is bent outwards in such a way that the antennæ projects laterally sweeping round the

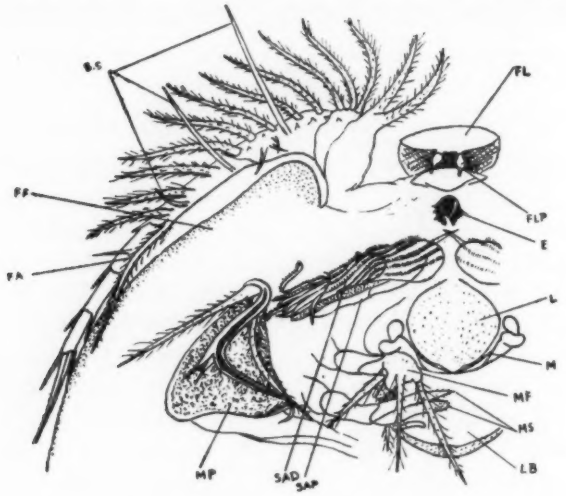

FIG. 2. *Bomolochus acuta* n. sp. Ventral view of head appendages × 150.

E.	Median eye.	*M.*	Mandible.
F.E.	Flexible flange of ventral plate.	*M.F.*	First maxilla.
		M.P.	Maxillipede.
F.A.	First antenna.	*M.S.*	Second maxilla.
F.L.	Frontal lobe.	*S.A.D.*	Second antenna distal part.
F.L.P.	Frontal lobe processes.	*S.A.P.*	Second antenna promimal part.
L.	Labrum.	*S.S.*	Sensory spines.
L.B.	Labium.		

anterior margin of the carapace. This joint, as well as the third and fourth joints, bear three simple sensory spines of varying lengths as well as eleven long plumose setæ on the anterior aspect of the appendage. Each plumose seta projects forwards and curves outwards to its sharp tip. In addition to these the fourth segment bears posteriorly on its outer edge, a long plumose seta. The fifth, sixth and seventh joints form the more slender, less armed distal part of the antenna. Each joint is of a tapering form and bears on its outer edge two short horizontal spines. The terminal joint bears two long setæ set close together. *The second antenna* is of two halves hinged and folded. The proximal two jointed part is attached behind the

base of the first antenna and is directed inwards so that the distal part is folded outwards. This part also consists of two joints of which one forms the bend or hinge. It is swollen, rounded and bears a short slender spine directed obliquely forwards and inwards. The fourth joint consists of a short cylindrical body to which a number of slender strips and about five long setæ hooked at their distal sharp tips are attached. These slender chitinous strips which are fused spiral-wise on the joint bear numerous teeth

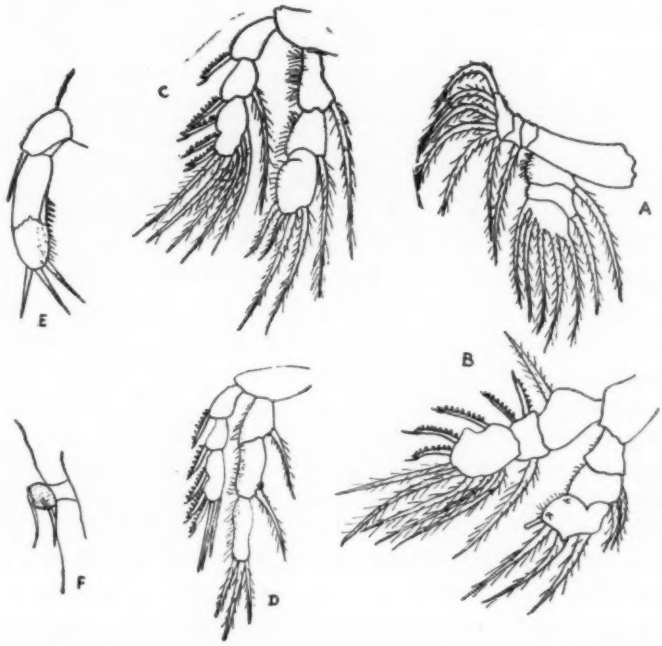

FIG. 3. *Bomolochus acuta* n. sp. Thoracic appendages (not drawn to scale).

A. First thoracic
B. Second thoracic
C. Third thoracic
D. Fourth thoracic
E. Fifth thoracic
F. Sixth thoracic (vestigial)

closely packed together and provide rough surfaces which help adhesion. Between the rounded inner ends of the second antennæ, the obovate labrum is attached. On either side of this upper lip in front of the labium are arranged three appendages on each side. Of these *the mandibles* are most anterior and closest to the labrum. It consists of a basal joint bent backwards, a middle joint with two rounded articular knobs at the base, as well as a distal cylindrical tapering portion. There is no palp and the terminal

joint is destitute of setæ. *The first maxillæ* are three jointed; a stout cylindrical muscular portion directed inwards, is followed by a similar second joint. The third part is bulbous and bears four long stiff plumose setæ directed backwards in different directions as well as a short setose palp-like body. *The second maxilla* consists of a two articled basal part and a bipartite distal part. The two rami are directed medially. One of these is sharp pointed while the other is blunt tipped, both being setose. *The maxillipedes* are three jointed. The basal joint is attached to the ventral surface of head behind the second maxillæ but is turned outside mouth parts in such a way that the rest of the appendage appears not only outside but also anterior to the other mouth parts. The second joint is roughly triangular in form, the inner proximal corner of which bears a slender sharp claw. The third joint is formed by a sigmoid curved stout claw directed backwards. This claw bears two sharp teeth at its second bend and ends in a sharp point. To the base of the claw are attached two plumose setæ one directed outwards and the other inwards.

Thoracic appendages.—The first are flattened and biramous. The basal joint is long. The exopod is three jointed and bears seven long plumose setæ; one on the second and six on the last joint. The endopod is also three jointed, the first and second joints bear one plumose seta each, while the third bears five plumose setæ. The second, third and fourth pairs of swimming feet also are biramous but are less flattened. In each leg the basal joint is single and supports both the exopod and endopod. The endopods are clearly three jointed while the exopods are really four jointed but, owing to the fusion of the last two joints, appear three articled. The joints are covered with hairs and bear a number of spines and setæ which are distributed as follows:—

II Endopod 0–1, 2–2, 1–3; II Exopod 0–1, 2–1, 3–6; III Endopod 0–1, 0–1, 2–2; III Exopod 1–0, 1–1, 3–5; IV Endopod 0–1, 0–1, 2–1; IV Exopod 1–0, 1–0, 3–3.

The fifth leg differs from the foregoing four in being uniramous as in all Ergasilids. They are smaller but are clearly three jointed. A well marked basal joint bears a long slender spine at its outer distal margin. The second joint is twice as long and bears a number of teeth on its inner aspect and the third joint is short, rounded, setose and bears three spines at its distal tip. The sixth leg is vestigial, consisting of a papilla and two slender spines.

Of the six thoracic segments the first has fused with the head to form the cephalothorax, the remaining five being distinct and free. The first of these is as broad as the cephalothorax but has a markedly convex hinder border

so that the next segment which is a third in width is articulated to its narrow posterior hump. The third segment resembles the second in being ellipsoidal but smaller being only two-thirds in width. The fourth segment is much narrower, being only half the breadth of the third. But its posterior edge being wider leads up to the slightly wider, genital segment. To the sides of this segment are attached the two long cigar-shaped egg sacs 1·32 mm. in length. These egg sacs are broadest at the anterior end being 0·17 mm. and taper posteriorly to a blunt point. Each egg sac contains about six to seven longitudinal rows of eggs, each row consisting of eighteen to twenty eggs.

The abdomen:—is three segmented and is of such a tapering form that the third segment narrows to nearly half the width of the first. Two anal laminæ, each bearing a short slender plumose seta on the outer side and a couple of spines on its distal margin are attached to the last segment. To the tip of each lamina are attached two long plumose setæ as long as the abdomen and anal laminæ put together. Of the two, the inner is slightly longer.

Systematic position.—This parasite clearly belongs to the genus *Bomolochus* (of the sub-family Bomolochinæ) since all the thoracic segments except the first are distinct and freely movable and the basal joints of the first antennæ are enlarged, flattened and densely armed. According to Wilson's artificial key, the maxillipedes being turned forward outside the other mouth parts, appears to be significant. The terminal claw on maxillipedes being sigmoid in shape and bearing two more teeth, the exopod of the first swimming leg having three joints of which the two terminal joints bearing plumose setæ and the free thoracic segments being of decreasing width, emphasise the resemblance to *B. exilipes* n. sp. Wilson. The present form, however, differs from this species in having only two plumose setæ (and not three) on the maxillipedes; the first maxilla having four plumose setæ (and not three); the first antenna being indistinctly segmented into seven and reaching far beyond the lateral margins; and the second antenna having shorter claws on its inner elbow. A comparison with forms hitherto recorded from the Indo-tropical area is made difficult owing to the incorrect descriptions and inaccurate sketches given by earlier authors. Of the species recorded from the Indo-tropical waters, the following eight species have been compared:—(1) *B. gracilis* found on *Zygæna malleus* Java (Heller, 1865), (2) *B. tetrodontis* found on *Tetrodon oblongus* Bombay (Bassett Smith, 1898), (3) *B. chætoessi* found on *Chetossus* sp. East Indies (Kroyer, 1863), (5) *B. unicirrus* found on *Amphisile scutata* Ceylon (Brian, 1902), (6) *B. megaceros* found on *Stromateus cinereus* Bombay, Colombo and on *Caranx djedaba*

Aden (Bassett Smith, 1898 and Heller, 1865), (7) *B. triceros* found on *Stromateus cinereus* Bombay (Bassett Smith, 1898), (8) *B. denticulatus* found on *Sphyræna jello* and *Hemirhampus far*. Of these two have been included by Wilson in a new genus Irodes the next three species have been removed to the new genus Artocolax (Wilson). The remaining three species differ from the present species in the following features. *B. denticulatus* differs in having the second free thoracic segment globose, longer than the first and bending the body at regular right angles; second antennæ having three terminal spines; the exopod of first leg being two jointed; the body being 3–4 mm. long; the egg sacs being as long as the whole animal and the frontal plate being semilunar, bearing two pedicles. *B. megaceros* is distinguished from the present species in being 4 mm. long; the first antenna having thirteen plumose setæ and three spines; the second antennæ bearing four spines; the sigmoid claw of the maxillipede bearing a single tooth; the second free thoracic segment being as broad as the first free thoracic segment; and in the number and distribution of the denticulate spines, simple spines and plumose setæ. *B. triceros* differs from the present form in possessing a very long pointed rostrum (not shown in figure); first swimming leg having a single jointed exopod bearing five plumose setæ and an endopod consisting of two joints; the second, third and fourth thoracic legs having no denticulate setæ and the anal lamina bearing a single strong bristle, two short lateral hairs, and a minute hair on outer border.

The present species can be distinguished from others in the following features. All free segments of the thorax except the first being distinct and uniform; a folded rostral plate bearing two processes; the first antenna having seven joints; the maxillipede bearing a sigmoid claw with two teeth and two plumose setæ; the exopod of second, third and fourth legs bearing denticulate spines, the exopod of first swimming leg being three jointed; the second antenna bearing five hooked spines; Hence the present species is described as a new species *Bomolochus acuta*. The type form will be lodged in the Indian Museum, Benares.

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AMIDE SYNTHESIS IN PLANTS

Part III. Urea Formation in Seedlings

By M. Damodaran, F.A.Sc., and T. R. Venkatesan

(From the University Biochemical Laboratory, Madras)

Received November 5, 1947

It was pointed out in the last paper in this series (Damodaran, *et al.*, 1946) that there were reasons for suspecting the formation, during seed germination and seedling growth, of a third amide in addition to asparagine and glutamine. Analysis of the changes taking place in amino-acids and amides in three species of germinating seedlings showed, in the first place, that the increase in the amide nitrogen in the non-protein fraction of the seedling extract was greater than could be accounted for by the increase in glutamine and asparagine; and secondly that in *Phaseolus mungo* and *Dolichos biflorus* in the later stages of seedling growth amide nitrogen was markedly in excess of the total dicarboxylic acids present. As urea has been shown to be widely distributed in fungi as well as in the higher plants (Wehmer, 1933) it was considered possible that this substance might be formed in seed germination also along with asparagine and glutamine.

In fungi urea is formed according to Iwanoff (1924, 1927) from ammonia and its function is to serve as a nitrogen reserve in the fruiting bodies. Klein and Taubock (1932) who studied the formation of urea in the higher plants were of opinion that it was derived from the hydrolysis of arginine and arginine-like compounds by the action of arginase. It was decided to test this hypothesis by carrying out in the course of the analysis described in the last paper simultaneous estimations of the quantities of urea and arginase present in the seedlings. As it has been shown (Damodaran and Sivaramakrishnan, 1937) that many leguminous seeds contain urease it was necessary to determine the amounts of this enzyme also as the quantity of urea present at any stage would represent the balance of the activities of arginase which hydrolyses arginine to give ornithine and urea and of urease which hydrolyses urea to carbon dioxide and ammonia.

Experimental

The present experiments were confined to *Dolichos biflorus* and *Phaseolus mungo* as these two were the seedlings which showed the greatest disparity between amide nitrogen and dicarboxylic acid content. The procedure with regard to germination of seedlings, preparation of samples for analysis and analytical methods for amino-acids and amides have been

described in the previous paper. Urea estimations were carried out every three days and urease and arginase activity every other day. The analyses of urea, urease and arginase were all reduced to the determination of ammonia the latter being carried out by the elegant micro-diffusion technique of Conway (1939).

Urea.—Urea estimations were made in the deproteinised aqueous extract of the seedlings prepared, as described previously, by removing the proteins by heat coagulation. Water melon urease prepared according to Damodaran and Sivaramakrishnan (1937) was used for the decomposition of urea. 1 ml. of standard N/70 sulphuric acid was pipetted out into the middle chamber of Conway's unit and a drop of Tashiro's indicator added. 1 ml. of the test solution was placed in the outside chamber followed by 0·5 ml. of urease (10% suspension in M/15 phosphate buffer at pH 7). The units after being closed airtight with discs smeared with grease (a mixture of paraffin and vaseline) were rotated slowly a few times to mix the enzyme and substrate solution completely and incubated at 37° for decomposition of urea. After 20 minutes 1 ml. of saturated potassium carbonate was added to the outer chamber. the solutions in the outside chamber mixed by gentle rotation as before and the apparatus left again in the incubator for two hours for absorption of ammonia by the acid. Excess of acid in the middle chamber was then titrated against CO_2-free N/70 sodium hydroxide using a Conway micro-burette. Preformed ammonia was determined in two control experiments in which the outer chamber of the Conway units contained (1) the enzyme urease only and (2) the solution under test only. The sum of the ammonia formed in the two determinations was deducted from that found in the experimental units. At least three units were used for each solution and the estimations discarded if not closely concordant.

Urease and arginase.—For preparation of extracts for determination of enzyme activities a constant number of uniformly grown healthy seedlings (usually thirty) was washed after removal of testa and after being lightly dried on filter-paper, thoroughly ground in an ice-cooled granite mortar. For urease estimations the macerate was brought to pH 7 with M/4 phosphate buffer and then made up to 25 ml. with M/15 phosphate buffer. For estimation of arginase the macerate was brought to pH 9·4 by gradual addition of 0·1 N sodium hydroxide and then made up to volume (25 ml.) with glycine-sodium hydroxide buffer of pH 9·4. 5 ml. aliquots were taken for estimation of enzyme activity.

Urease activity determination was carried out by taking 5 ml. of the suspension at pH 7, 5 ml. of urea solution (M/2 when activity was high and

M/10 in other cases) and phosphate buffer at pH 7 to make upto 25 ml. and allowing them to react at 37° for exactly twenty minutes after which period the enzyme was inactivated by the addition of 5 ml. of N-sulphuric acid and keeping at 80° for ten minutes. The solution was made upto volume and ammonia determined on an aliquot in the Conway apparatus. A control experiment using boiled seedling extract was carried out at the same time. The total N in the suspension was determined in each case and urease activity expressed as the number of mgm. of urea N decomposed per 100 mgm. total nitrogen in 20 minutes.

For determination of arginase activity the following procedure was adopted. 5 ml. of the suspension under test, 5 ml. of M/100 arginine solution (shown by preliminary experiments to provide excess substrate) both at pH 9·4 were mixed and made up to 25 ml. with sodium hydroxide-glycine buffer at pH 9·4. The reaction mixture was allowed to stand at 37° for three hours under a layer of toluene. At the end of the period the enzyme was inactivated as before by keeping at an acid reaction at 80° for 10 minutes. The solution was then brought to pH 7 by addition of N-sodium hydroxide and urea in an aliquot determined by the method described above. A control was conducted as usual with boiled enzyme. Arginase activity was expressed as the number of mgm. urea N formed from arginine in three hours.

Results

Table I

Dolichos biflorus *seedlings*

Values expressed as percentage of N of seedlings: Enzyme units per 100 mgm. of seedling N

Age of seedlings in days	1	3	4	5	7	9	10	11	13	15	16	17	19	22
Amide ..	9·8		18·9		25·1		29·2		31·8		34·8		32·7	33·8
Dicarboxylic acids ..	21·4		24·7		25·6		25·6		24·6		22·9		23·9	23·4
Excess amide over dicarboxylic acids							3·6		7·2		11·9		8·8	10·4
Urea ..	0·3		0·5		0·8		2·8		6·9		13·0		16·0	11·0
Arginine ..	12·4		8·2		7·1		4·3		2·8		2·3		3·3	2·6
Fall in arginine ..			4·2		5·3		8·1		9·6		10·1		9·1	9·8
Arginase units ..	0·9	6·5		10·9	14·1	22·7		47·6	58·1	64·4		59·3	63·6	
Urease units ..	527	477		215	13·7	4·9		4·7	2·9	2·2		3·8	3·4	

TABLE II

Phaseolus mungo *seedlings*

Values expressed as percentage of N of seedlings: Enzyme, units per 100 mgm. of seedling N

Age of seedlings in days	1	3	4	5	7	9	10	11	13	15	16
Amide ..	11·2		20·3		29·0		31·7		32·5		35·1
Dicarboxylic acids ..	23·1		28·0		32·4		28·4		28·5		29·3
Excess amide over dicarboxylic acids							3·3		4·0		5·8
Urea ..	0·2		1·3		3·4		8·5		8·6		3·5
Arginine ..	13·9		12·0		11·3		11·6		10·6		10·9
Fall in arginine ..			1·9		2·6		2·3		3·3		3·0
Arginase units ..	6·6	1·5		2·0	3·5	0·8		0·4	4·1	1·1	
Urease units ..	4·3	0·14		0·27	0·5	0·56		0·35	0·56	0·63	

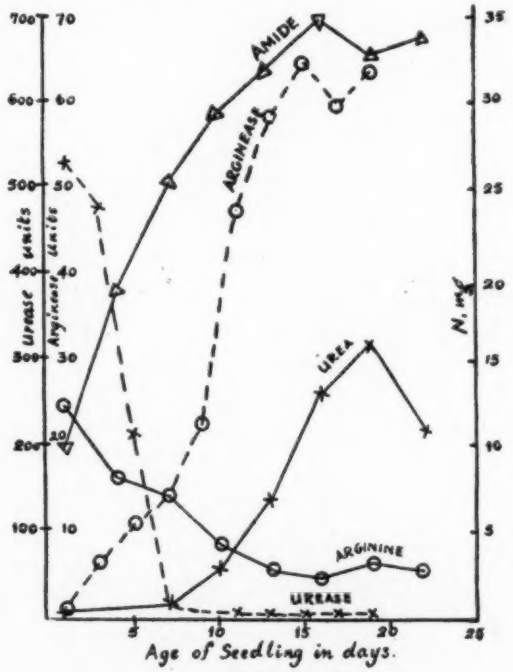

FIG. 1. *Dolichos biflorus*

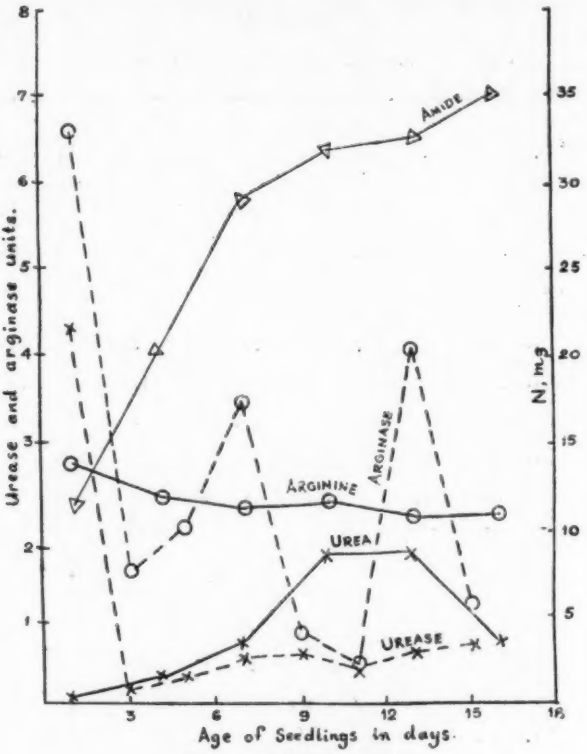

FIG. 2. *Phaseolus mungo*

DISCUSSION

It is clear from the results obtained that formation of urea is one of the important chemical changes taking place during seed germination and reaches quite considerable proportions. Thus in the nineteen-day old seedlings of *Dolichos biflorus* the amide nitrogen is 32·7% and the urea 16·0% or nearly half of the total amide present. In *Phaseolus mungo* urea production reaches its maximum on the 13th day. At this stage amide nitrogen is 32·5% and urea 8·6% or 28% of the total amide. The quantity of urea produced is more than sufficient to account for the excess of amides over dicarboxylic amino-acids and explains this discrepancy.

The origin of the urea is, however, not so clear. In *D. biflorus* there is reason to conclude that arginine is the precursor of at least part of the urea found. There is a considerable and rapid decline in the quantity of the arginine present while simultaneously there is a parallel change in enzyme

activity in the seedling, the urease activity showing a sharp fall and the arginase activity a steady increase (Fig. 1). But as only half the arginine N can appear as urea the fall in arginine, as the figures in Table I show, is insufficient to account for the whole of the urea formed. In *P. mungo* the proportion of the urea that could be formed from the arginine that has disappeared is still smaller. There is no great decrease in the arginine content of the seedling throughout the experimental period; the arginase activity is also low and shows no such steady rise as in *D. biflorus* (Fig. 2). The hydrolysis of arginine cannot therefore account for more than a fraction of the urea formed. Klein and Taubock were of opinion that urea arose from arginine-like compounds such as canavanine. An examination of several leguminous seeds made in this laboratory for canavanase in connection with a former investigation (Damodaran and Narayanan, 1940) however, gave no indication for the presence of this amino-acid in the seed reserves of either *P. mungo* or *D. biflorus*. While the presence of other unknown precursors of urea cannot be excluded a more plausible hypothesis for the formation of urea (other than that arising from arginine) would appear to be by synthesis from ammonia and carbon dioxide either directly by the action of urease or indirectly *via* the Krebs ornithine cycle. Further experimentation is required to test the validity of any such hypothesis.

Summary

It has been demonstrated that the formation of urea is one of the important biochemical changes taking place in the course of protein regeneration in seedlings. The quantities of urea formed in *Dolichos biflorus* and *Phaseolus mungo* represent a considerable proportion of the amides formed in these seedlings and explain the observation previously reported that amides are in excess of the dicarboxylic acids.

From the quantitative relations between the changes in urea, urease, arginine and arginase it is inferred that at least in *Dolichos biflorus* a part but not the whole of the urea arises from the hydrolysis of arginine. It is, however, necessary to assume the existence both in *Dolichos biflorus* and *Phaseolus mungo* of an additional mechanism responsible for urea formation.

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ERRATA

Vol. XXVI, No. 4, Section B, October 1947

On Page 145, first line, *H. cannabinos* should read *H. cannabinus.*

In the list of References against 15, *read* Thomas, Ramakrishnan, Soumini and Balakrishnan, *Proc. Ind. Acad. Sci.*, (B), 1947, **26**, 147–163 instead of *Proc. Ind. Acad. Sci.*, (B), 1947, **25** (in Press).

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ADDITIONS TO FUNGI OF MADRAS—IV*

By T. S. Ramakrishnan and K. Ramakrishnan
(*Mycology Section, Agricultural Research Institute, Coimbatore*)

Received September 10, 1947
(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

(16) *Bulgariastrum tumifaciens* Ramakrishnan T. S. and K. sp. nov.

On pedicels and young fruits producing dark gall-like outgrowths; on leaves forming thickened, brown, isolated to confluent spots; *apothecia*, amphigenous on the leaves, clustered, sub-turbinate, black, disc-shaped, slightly concave at the top, with short stout stalks, 400–700 μ in diameter; *asci* cylindric to clavate, narrowed at the base, hyaline 80 × 12 μ (65–115 × 10–17 μ), 8-spored, paraphysate, paraphyses branched at the tip, forming thick-walled dark brown cells which become compacted into a dark epithecium; *ascospores* elliptical, two-celled, constricted at the septum, hyaline, 13 × 5·4 μ (10–19 × 4·8–9·6 μ), more or less uniseriate; *pycnidia* mixed with the apothecia more numerous on the galls on the inflorescence, often developing on the stalks of the apothecium, flattened, immersed in the stroma, or on the surface, covered by arching, black layers of cells which rupture easily and expose the pycnidiospores; sometimes the ruptured pycnidia resemble acervuli; *pycnidiospores* produced on short stalks, 2-celled, fusiform to clavate, light olive, lower cell usually more elongated than the upper cell, 18 × 7 μ (15–22 × 4–9 μ).

On living leaves and inflorescence of *Capparis sepiaria* L. Hasanur (Coimbatore District) 16-8-1946, C. R. Venkataraman and Kallar (Coimbatore District) 9-10-1946, T. S. Ramakrishnan and K. Ramakrishnan.

Ascomata amphigena, in incrassatis maculis foliorum, gregaria, turbinata, nigra, brevistipitata, 400–700 μ diam. *asci* cylindrici vel clavati, hyalini, octosporiati, 80 × 12 μ (65–115 × 10–17 μ), *ascosporidia* duocellata, hyalina, oblique uniseriata, paraphysata 13 × 5·4 μ (10–19 × 4·8–9·6 μ), termini paraphyses ramifacti, rami juncti in fuscum epithecium; *pycnidia* in excrescentiis formata in inflorescentiis et fructibus, etiam ascomatibus mixta, cum irregulari tecto, quod erumpit variæ; *pycniosporidia* duo cellata, clavata levis olivacei colores.

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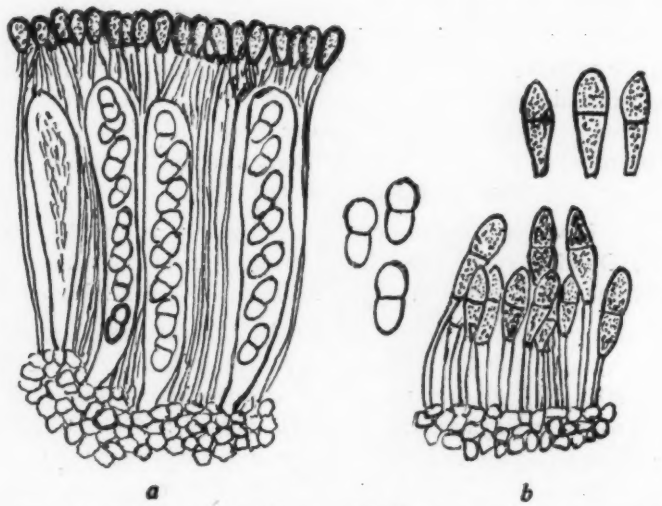
* 1947, Part III of this series was published in the *Proceedings of the Indian Academy of Sciences*, **26**, No. 1.

Type specimens of all the fungi described have been deposited in the Herbarium of the Government Mycologist, Coimbatore, and the *Herb. Crypt. Ind. Orient.*, New Delhi.

In vivis foliis et inflorescentiis *Capparidis sepiariæ* L. Hasanur (Coimbatore District) 16-8-1946, C. R. Venkataraman et Kallar (Coimbatore District) 9-10-1946, T. S. Ramakrishnan et K. Ramakrishnan.

Bulgariastrum cæspitosum Syd. has been recorded from the Philippines on *C. sepiaria*. But this fungus forms ascomata on the lower surface of the leaves. Further the pycnidia produce hyaline spores measuring 26–45 μ. The fungus under study produces galls on the inflorescence also. The pycnidiospores are coloured and measure only 15–22 μ, and thus it is different from the former. Dr. Mundkur to whom the type specimen was sent for comparison with *B. cæspitosum*, available at the Herbarium Cryptogammæ Indiæ Orientalis, New Delhi, also found that the fungus under study was different. *B. africanum* Syd. has been recorded on *C. rudatis* from Natal. This fungus affects leaves only, producing ascomata, on both sides of the leaf. But it does not produce galls on the inflorescence. The pycnidial stage of this species has not been observed. For these reasons the present fungus is considered to be a new species and named *B. tumifaciens*.



TEXT-FIG. 1. *Bulgariastrum tumifaciens*.—(*a*) Section through a portion of apothecium showing asci and paraphyses; (*b*) Pycnidiospores (× 600).

(17) *Achroella plectroniæ* Ramakrishnan T. S. and K. sp. nov.

Stromata black, pulvinate, glomerulate, erumpent, amphigenous, developed in the middle of a circular, thickened, brown spot which forms a ring-like structure round the stroma, sparse or crowded, sessile attached by a

broad base to the leaf-tissue, subepidermal in origin; *perithecia* half immersed in the stroma, with a black outer wall, ovate to globose, ostiolate, 240 × 170 μ, paraphysate, with fine filiform paraphyses; *asci* clavate, hyaline, with gelatinising wall, rounded at the ends and with a short narrow foot, 82 × 15 μ (72–91 × 14–19 μ); *ascospores* 8, fascicled, fusoid, tapering towards the ends, one-septate, light yellowish brown, 44 × 3 μ (38–50 × 2·5–3·5 μ).

Pycnidia of two kinds, one type resembling *Hemidothis*, developed in the stroma, associated with the perithecia or not, loculi sunk in the stroma, ostiolate; pycnidiospores long fusoid, curved, hyaline, one-celled, 63 × 3·5 μ (40–76 × 2–4·5 μ), produced on short hyaline stalks. The second type of pycnidium forms isolated, crowded, innate, erumpent, subepidermal, globose, ostiolate, dark structures; spores numerous, minute, spermatia-like; obviously these pycnidia function as spermogonia.

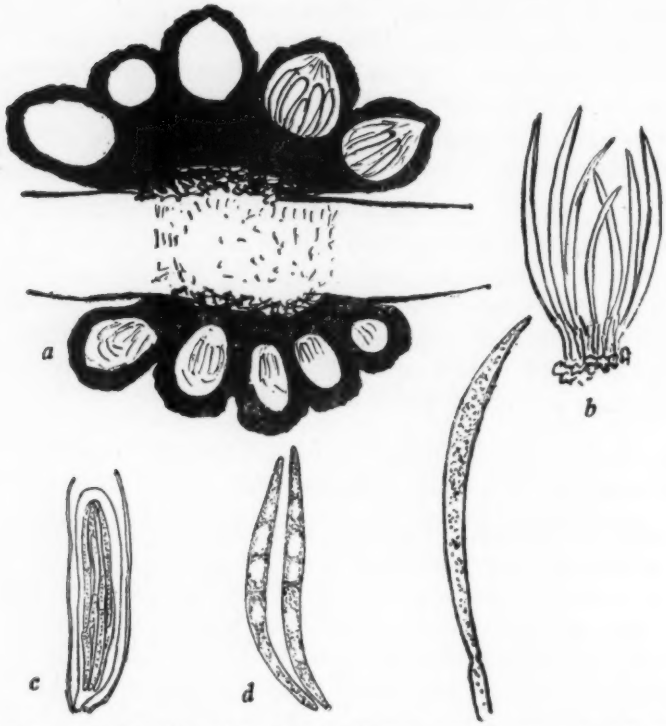
On living leaves of *Plectronia didyma* Kurz. Kallar (Coimbatore District), November 1946. T. S. Ramakrishnan.

Maculæ amphigenæ, orbiculares, incrassatæ; stromata nigra, glomerulata, amphigena; *perithecia* pars dimidia immersa, muris externi nigris, ovata vel globosa, ostiolata, 240 × 170 μ, paraphysata, paraphyses filifirmes; *asci* clavati, hyalini, murus asci gelatinus, 82 × 15 μ (72–91 × 14–19 μ); *ascosporidia* 8, fasciculata, fusoidea, angustata ad terminum, uniseptata, levis brunnei colores, 44 × 3 μ (38–50 × 14–19 μ).

Duo genera *pycnidium*, (1) sociata cum perithecia, immersa, ostiolata; *Pycnidiosporidia* longa, fusoidea, curvata, hyalina, unicellata, 63 × 3·5 μ (40–76 × 2–4·5 μ), pedicelli hyalini, brevi, (2) isolata amphigena, gregaria, innata, erumpentia, subepidermia, globosa, ostiolata, nigra; pycnidiosporidia numerosa, minuta, hyalina, baculoformia.

In vivis foliis *Plectroniæ didymæ* Kurz. Kallar (Coimbatore District), November 1946, T. S. Ramakrishnan.

The *Hemidothis*-like pycnidia are the most conspicuous. Perithecia are rare. Sydow (1916) in his description of *Hemidothis* has stated that it represents only the imperfect stage of a Dothidiaceous ascomycete, probably *Bagnisiopsis* or some related genera. In the present instance the perfect stage is represented by a fungus belonging to the Dothidiaceæ. Considering the structure of the stroma, asci and ascospores it has to be identified as *Achroella*. No allied fungus has been recorded on the present host. This fungus is considered to be a new species and named *A. plectroniæ*.



TEXT-FIG. 2. *Achroella plectroniæ*.—(*a*) Section through stromata (diagrammatic); (*b*) Pycnidiospores (×500–1000); (*c*) ascus and paraphyses (× 300); (*d*) ascospores (× 500).

(18) ***Hysterostoma pavettæ*** Ramakrishnan T. S. and K. sp. nov.

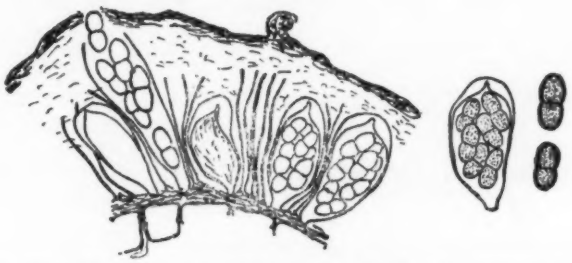
Spots yellowish, amphigenous, circular; stromata superficial, epiphyllous, dark; hypostroma subcuticular; haustoria sent into the epidermal and palisade cells, the tissue of the stroma gelatinises when moistened; asci stout, club-shaped, with rounded apex, and narrow base, wall-thickened and gelatinising near the top, 50 × 24 μ (40–59 × 19–33 μ), paraphysate, paraphyses fine, filiform; *ascospores* 8, smoky brown, 2-celled, oblong, constricted at the septum, 21 × 9 μ (18–26 × 7–11 μ).

On living leaves of *Pavetta indica* L. on the road from Kallar to Burliar (Coimbatore District), November 1946, T. S. Ramakrishnan.

Maculæ amphigenæ, flavidæ, orbiculares; stromata superficialia, epiphylla, fusca, hypostroma subcuticulare, haustoria penetrantia in cellæ epidermes et paliformes; stroma gelatinum in aqua; *asci* crassi, clavati,

apice rotundato, basi attenuato, murus incrassatus et gelatinans ad summum, 50 × 24 μ (40–59 × 19–33 μ), paraphysata, paraphyses fragiles, filiformes; *ascosporidia* 8, fumide brunnei colores, duo cellata, oblonga, medio constricta, 21 × 9 μ (18–26 × 7–11 μ).

In vivis foliis *Pavettæ indicæ* L. in via de Kallar ad Burliar (Coimbatore District), November 1946, T. S. Ramakrishnan.



TEXT-FIG. 3. *Hysterostoma pavettæ*.—Stroma, ascus and ascospores (× 300).

This genus has not been recorded on this host so far from India or elsewhere. It is described as a new species. Hansford (1946) has described several species of this genus on various hosts but there is no mention of *Pavetta* among them.

(19) *Phæochorella artocarpi* Ramakrishnan T. S. and K. sp. nov.

Stromata amphigenous on the leaves, sometimes present on the petioles also, but more pronounced on the upper side along the midrib and main veins forming long raised areas; on the lower surface stromata less prominent and occupying corresponding positions or otherwise, black shiny, subcuticular, and made up of closely packed, dark brown, vertical hyphæ; location of the perithecia indicated by conical raised projections on the upper surface, loculi conical and much flattened, 170 × 86 μ; *asci* cylindric, clavate, 8-spored, hyaline, 65 × 16 μ (52–89 × 11–22 μ) paraphysate; *ascospores* light olive when young, and dark brown with age, elliptic, one-celled, uniseriate, 14 × 7 μ (11–17 × 4–9 μ); *pycnidia* found in the stromata on the lower surface of the leaf, ostiolate, of varying dimensions, immersed in the stromata or not, *pycnidiosporidia* oval to elliptic, one-celled, light olive, 5·5 × 4 μ (4·5–7 × 3–5 μ).

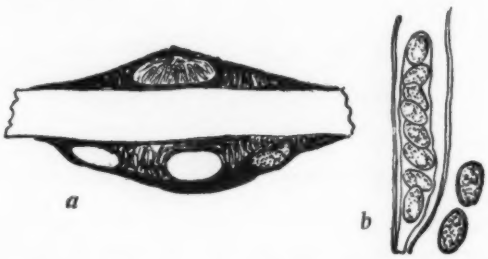
On living leaves of *Artocarpus Lakoocha* Roxb. Burliar (Nilgiris), November 1946, T. S. Ramakrishnan.

Stromata amphigena, mediam venam et prominentes venas sequentia, extanter epiphylla, nigra, micantia, sub-cuticularia, composita hyphis

verticalibus compactis; *perithecia* plurimum epiphylla, ostiolata, compressa, 170 × 86 μ; *asci* clavati, cylindrici, octosporiati, hyalini, paraphysati, 65 × 16 μ (52–89 × 11–22 μ); *ascosporidia* levis olvacei colores in juventute, fusci brunnei colores in maturitate, elliptica, unicellata, uniseriata, 14 × 7 μ (11–17 × 4–9 μ).

Pycnidia hypophylla, immersa in stromata; *pycnidiosporidia* ovalia vel elliptica, unicellata, levis olivacei colores, 5·5 × 4 μ (4·5–7 × 3–5 μ).

In vivis foliis *Artocarpi Lakoochæ* Roxb. Burliar (Nilgiris), November 1946, T. S. Ramakrishnan.



TEXT-FIG. 4. *Phæochorella artocarpi.*—(*a*) Section through stromata (diagrammatic); (*b*) ascus and ascospores (× 300).

Catacauma microcentum (Berk. and Broome) Theiss. and Syd. has been recorded on *Ficus mysorensis*. The present fungus produces subcuticular stromata with closely packed vertically disposed hyphæ, but it differs from *Catacauma* in having ascospores which are coloured when mature. It is therefore placed in the genus *Phæochorella*. This genus has not been recorded on this host and the fungus is consequently named *P. artocarpi*. It is interesting to note that the pycnidial stage is confined to the lower surface of the leaf and the ascus stage to the upper surface. The tissues bordering the stromata sometimes develop a brown discolouration. Hyphal connection between the lower and upper surfaces of the leaf through the tissues is indistinct.

(20) *Puccinia luculenta* (Syd.) Ramakrishnan T. S. and K. comb. nov.

Pycnia not present; *æcia* sunk in the woody galls produced on both sides of the leaves; galls vary in size from 0·75–6 mm., almost spherical, dark brown, with varying numbers of æcia sunk in each gall; æcia present on both sides; each æcium has a definite peridium made up of one layer of thick-walled rectangular to polygonal warty cells; *æciospores* globose or angular with a coarsely verrucose unequally thickened, hyaline wall,

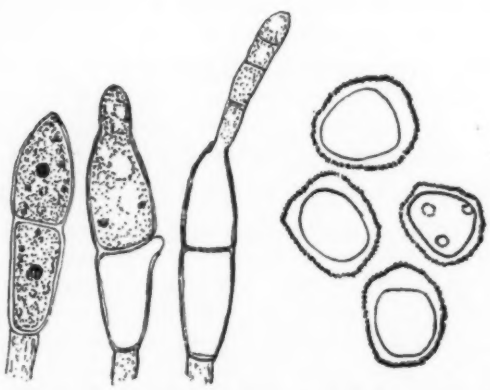
catenulate, germpores two or more, clearly visible in younger spores. 40 × 35 μ (34–48 × 29–38 μ), orange coloured.

Uredia not observed; *telia* amphigenous, waxy, erumpent, cushiony, originating 2 to 3 layers below the epidermis which is burst through. The teliospores originate from a compact plectenchyma; each spore is stalked with a prominent, gelatinising, hyaline pedicel up to 200 μ in length; *teliospores* 2-celled, cylindrical, with tapering ends, slightly constricted at the septum, wall smooth, contents orange yellow in colour, with one germpore in each cell; germpore in the upper cell at the apex while the germpore in the lower cell is just below the septum; spores measure 77 × 25 μ (58–106 × 19–34 μ), germinate immediately.

On living leaves of *Loranthus longiflorus* Desv. Attakatti (Anamalais), 10–4–1947, T. S. Ramakrishnan.

Pycnia ignota; *æcia* demersa in globosis amphigenis, lignieis excrescentiis; peridium unius strati cellarum polygonarum, crassomuratarum, echinularum; *æciosporidia* globosa vel angularia, catenulata, exstanter verrucosa, murus inequaliter incrassatus, aurantiacei colores, porum germinationis unum vel plura; *uredia* absunt; *telia* amphigena, ceracea, erumpentia, pulvinata, subepidermia; *teliosporidia* stipitata, cum pedicellis extanter gelatinantibus, hyalinis usque 200 μ longa, teliosporidia duocellata, cylindrica, angustatis terminis, medio leviter constricta, episporio levi, contenta aurantiacei flavi colores, quaque loculo porum germinationis unum gerente 77 × 25 μ (58–106 × 19-34 μ) germinantia *in situ*.

In vivis foliis *Loranthi longiflori* Desv. Attakatti (Anamalais), 10–4–1947, T. S. Ramakrishnan.



TEXT-FIG. 5. *Puccinia luculenta*—teliospores and æciospores (× 300).

Several rusts have been observed on *Loranthus*. From Mysore Butler and Bisby (1931) have recorded *Aecidium luculentum* Syd. of which only æcia were observed on leaves and stem of *L. longiflorus*. *Aecidium Schimperi* Bacc. has been observed on *L. Schimperi* from tropical Africa. On *Loranthus crassipes* and *Loranthus* sp., *Uromyces socius* Arth. and Holw. has been recorded from Central America. McAlpine (1906) has described *P. loranthicola* McAlp. on *L. celastroides*, from Australia. *P. loranthi* Speg. on *L. cordatus* and *P. macrocarya* Racib. on *L. pentandra* have also been reported. Cummins (1941) has recorded *P. heroica* Cumm. and *P. macrocarya* on *Loranthus* spp. from New Guinea. *P. loranthicola* produces æcia, uredia and telia. But æcia are found on separate leaves. The teliospores are intermixed with the urediospores. In these it differs from the rust under study, which does not form uredia, and the æcia and telia are formed on the same leaf. *P. loranthi* forms only telia and the spores are smaller than those of the present rust. *P. macrocarya* and *P. heroica* form pycnia. The teliospores are yellowish (or nearly hyaline in *P. macrocarya*) and the pedicels are concolorus. Spots are formed round the hypophyllous telia of *Puccinia heroica*. In the rust under study pycnia have not been found. The telia are amphigenous and no spot is visible surrounding the telium. The stalks of teliospores are hyaline while the teliospores are orange yellow in colour. Further, the stalks gelatinise readily when moistened. In these characters it differs from both the above species. A comparative statement of these rusts is given below.

	Aeciospores	Teliospores	Remarks
P. heroica ..	35–48 × 45–65 μ	69–26 × 18–23 μ	Pycnia present, æciospore with apical thickening, telia hypophyllous, teliospores yellowish, with concolorous stalks.
P. macrocarya ..	33–39 × 39–46 μ	72–89 × 23–30 μ	Pycnia prominent; æcia mostly hypophyllous, telia amphigenous, teliospores yellowish with concolorous stalks.
P. luculenta ..	34–48 × 29–38 μ	58–106 × 19–34 μ	Pycnia absent, æciospore with unequally thickened wall with 2 or more germpores, telia amphigenous, teliospores, orange yellow, pedicel hyaline and gelatinising

Butler and Bisby (1931) have described *A. luculentum* on *Loranthus longiflorus* from Mysore. The æcia of the rust under study resemble those of *A. luculentum*. Since the perfect stage has now been noticed on the same host mixed with the æcia a new combination *P. luculenta* is proposed.

The gelatinisation of the stalks of the teliospores is very prominent and resembles what occurs in *Gymnosporangium*. Germpores are visible in the young æciospores. These characters have not been noticed in the other species of *Puccinia* recorded on this host.

(21) *Hapalophragmium anamalaiensis* Ramakrishnan T. S. and K. sp. nov.

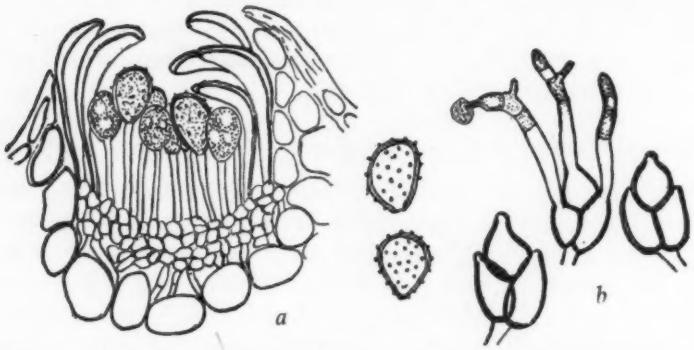
Pycnia and *æcia* not known; *uredia* hypophyllous, minute, scattered, light brown, erumpent, subepidermal in origin; *urediospores* stipitate, oval to elliptic, brown to orange, echinulate, 34 × 23 μ (24–38 × 19–27 μ), paraphysate, paraphyses incurved light brown, thickened on the outer side at the upper portion and not on the inner side, forming a protective covering for the sorus; *telia* hypophyllous, darker in colour than the uredia, minute, erumpent, scattered, subepidermal, in origin, paraphysate, paraphyses like those of the uredia; *teliospores* stipitate, stalk hyaline; rhomboidal in shape, three-celled, with two lower cells and one upper; stalk attached at the base of the two lower cells at the indentation; the two lower cells are elongated and are in contact for half their lengths, the top cell is wedged in between these, and rhomboidal in shape; wall smooth and thin, light brown in colour, with one germpore at the tip of each cell; spores measure 43 × 28 μ (35–58 × 17–34 μ), germinate immediately without a period of rest, producing one hyaline promycelium from each cell; promycelium coming out through the germpore, four-celled with one basidiospore from each cell.

On living leaves of *Derris eualata* Bedd. Anamalais, 10–4–1947, T. S. Ramakrishnan.

Pycnia et *æcia* ignota; *uredia* hypophylla, minuta, levis brunnei colores, subepidermia; *urediosporidia* stipitata, ovata vel elliptica, brunnei vel aurantiacei colores, echinulata 34 × 23 μ (24–38 × 19–27 μ), paraphysata; paraphyses incurvatæ, levis brunnei colores, externæ incrassatæ; *telia* hypophylla, fusciora quam uredia, minuta, erumpentia, subepidermia, paraphysata ut urediis; *teliosporidia* stipitata, rhomboidea, 3-cellata, 2 infra et una supra, unum porum germinationis in quaque cella in apice, brunnei colores, 43 × 28 μ (35–58 × 17–34 μ), germinantia sine quiete.

In vivis foliis *Derridis eualatæ* Bedd. Anamalais, 10–4–1947, T. S. Ramakrishnan.

H. derridis Syd., *H. setulosum* (Pat.) Syd., and *Triactella pulchra* (Rac.) Syd., have been recorded on *Derris uliginosa*, *Derris* sp., and *D. elliptica*, respectively. Sydow and Petrak (1931) are of opinion that *T. pulchra* has to be renamed *H. pulchrum* and that the genus *Triactella* which was founded



TEXT-FIG. 6. *Hapalophragmium anamalaiensis*. (*a*) uredium and urediospores (× 300); (*b*) teliospores (× 300).

on *T. pulchra* has to be abandoned. The rust under study is a *Hapalophragmium*, but it differs from the previously recorded species in several respects. *H. setulosum* (Miles and Traverse, 1904) and *H. pulchrum* (Sydow and Petrak, 1931) have warts near the germpores of the teliospores, whereas in the rust under study the teliospores are perfectly smooth. The sori of *H. derridis* are amphigenous and paraphyses have not been recorded. In the rust under study, however, the sori are hypophyllous and numerous characteristic paraphyses are present. For these reasons this rust is found to be different and is, therefore, named *H. anamalaiensis*.

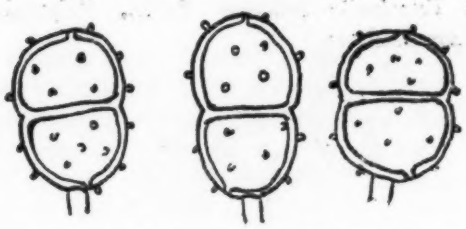
(22) *Puccinia vernoniæ-monosis* Ramakrishnan T. S. and K. sp. nov.

Pycnia, *æcia* and *uredia* absent; *telia* hypophyllous, sparse, erumpent, sub-epidermal, cushion-shaped, with the leaf thickened at the affected region, dark brown in colour; *teliospores* stipitate, with a short hyaline stalk, 2-celled, elliptic to subglobose, rounded at the apex and base, thick-walled, prominently echinulate, with sparse hyaline echinulations, reddish brown in colour, 35 × 27 μ (29–43 × 24–34 μ), germpore one in each cell.

On living leaves of *Vernonia monosis* C. B. Clarke, Valparai (Anamalais), 10–4–1947, T. S. Ramakrishnan.

Pycnia æcia et *uredia* non cognita; *telia* hypophylla, sparsa, erumpentia, fusca; *teliosporidia* stipitata, duocellata, elliptica vel sub-globosa, exstanter sparse echinulata, rubrei brunnei colores, 35×27 μ (29–43 × × 24–34 μ).

In vivis foliis *Vernoniæ monosis* C. B. Clarke, Valparai (Anamalais), 10–4–1947, T. S. Ramakrishnan.



TEXT-FIG. 7. *Puccinia vernoniæ-monosis.*—Teliospores (× 600).

The host plant is arboreal in habit and represents one of the three tree species of *Vernonia* in South India. Jackson (1918, 1932) has given a list of *Puccinia* occurring on species of *Vernonia*. Over fifty species of *Puccinia* have been included in this list. These have been classified into two groups, having smooth or rough-walled teliospores. The rust under study is a microform having only the III stage. It has teliospores with prominent sparsely placed echinulations and is different from other recorded species. Further no *Puccinia* has been recorded on the present host. Therefore, the rust is considered a new species and named *Puccinia vernoniæ-monosis*.

(23) *Aecidium terminaliæ* Ramakrishnan T. S. and K. sp. nov.

Spots amphigenous, sparse, yellow to reddish brown, with a distinct more or less spherical, woody gall in the centre; the gall projects out on both sides of the leaf; sometimes the galls are formed along the margins of the leaves also. *Pycnia* amphigenous, subcuticular hemispherical; *pycniospores* hyaline, spindle-shaped; *æcia* sunk in the woody galls, varying numbers of cupulate hard-rimmed æcia present in each gall, æcia measure 670 × 360 μ; peridium ephemeral, made up of polygonal smooth-walled cells; *æciospores* catenulate, polyhedral, with thick walls, one end sometimes thicker than the other, densely and prominently verrucose, measuring 42 × 24 μ (29–49 × 19–29 μ), deep reddish brown.

On living leaves of *Terminalia bellerica* Roxb., Valparai, 10-4-47, T. S. Ramakrishnan.

Maculæ sparsæ, amphigenæ, brunnei colores, cum distincto, spherico, ligneo, excrescente projiciente ambabus lataribus; *pycnia* amphigena, subcuticularia, hemispherica; *pycniosporidia* hyalina, fusiformia; æcia immersa, cupulata, 670 × 360 μ, peridii evanescentis, cellulis polygonis, pariete levi; *æciosporidia* catenulata, polyhedra, interdum inequaliter incrassata, exstanter et dense verrucosa, 42 × 24 μ (29–49 × 19–29 μ), dense rubrei-brunnei colores.

In vivis foliis *Terminaliæ bellericæ* Roxb., Valparai (Anamalais), 10–4–1947, T. S. Ramakrishnan.

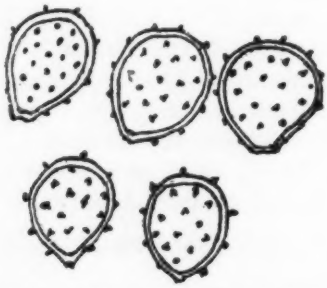
TEXT-FIG. 8. Aecidium terminaliæ æciospores (× 300).

The only rust recorded on this host genus is *Uredo terminaliæ* P. Henn. on *T. argentea* and *T. hyalobotes*. In the fungus now collected only the æcial stage is observed and it is therefore for the present included under the form genus *Aecidium* and is named *A. terminaliæ*.

(24) *Uredo amomi* Petch

Spots small, yellowish brown, isolated amphigenous; *uredia* hypophyllous, minute, pulverulent, one or more in a spot, whitish, subepidermal; *urediospores* ovate, globose, strongly spinulose, stipitate, hyaline to light yellow, 28 × 24 μ (24–38 × 19–29 μ).

On living leaves of *Amomum* sp., Valparai (Anamalais), 10–4–1947, T. S. Ramakrishnan.



TEXT-FIG. 9. *Uredo amomi*.—Urediospores (× 600).

A similar rust was prevalent on the leaves of *Elettaria cardamomum* on the Anamalais during the same period. Yellowish brown spots are visible on the upper surface. Minute uredia develop in clusters on the lower surface of the spots. The uredia are whitish to pale brown in colour. Urediospores are oval to globose, strongly spinulose, hyaline to light yellow and measure 22–32 × 20–28 μ.

A comparison of the above two rusts exhibited complete agreement in all characters.

Phakopsora elettariæ (Rac.) Cumm. has been recorded on *Amomum* sp. from New Guinea. Cummins (1941) had concluded that the description of the telia of *Schroeteriaster elettariæ* Rac. indicated that it was really a *Phakopsora*. *S. elettariæ* was recorded on *Elettaria cardomomum* by Raciborski 1900. *Uredo amomi* Petch has been described from Ceylon (Petch, 1911–14) on *Amomum involucratum*. A comparative statement of *P. elettariæ*, *U. amomi* and the two present rusts is given below.

	Urediospore dimensions	Remarks
P. elettariæ ..	21–30 × 15–20 μ	Orange yellow, finely verruculose
U. amomi ..	25–38 × 20–28 μ	Strongly spinulose, hyaline
Rust on *Amomum* ..	24–38 × 18–29 μ	Hyaline to light yellow, strongly spinulose
Rust on *Cardamomum* ..	23–32 × 20–28 μ	Hyaline to light yellow, strongly spinulose

It is evident from the above statement that the present rusts are identical with *U. amomi* in spore size, and shape and in the nature of the wall. Some of the spores in the present rusts have a light yellowish colour, which however cannot be taken as a valid differentiating character. It is very probable that all the three rusts represent only the uredial stage of *P. elettariæ*. But since only the uredia have been seen they are tentatively placed in *U. amomi*.

(25) *Uredo colebrookiana* Barclay.

On leaves of *Colebrookia oppositifolia* Sm., Valparai (Anamalais), 10–4–1947, T. S. Ramakrishnan.

This rust has not been observed in South India before.

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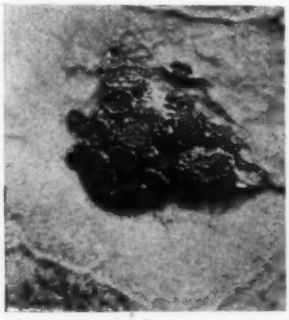
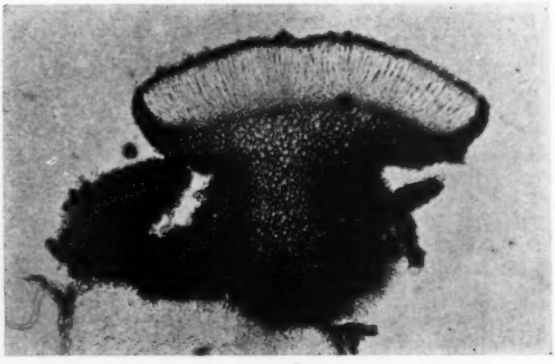


2



3

Bulgariastrum tumefaciens

1. Leaves and inflorescence of *Capparis sepiaria*, showing galls
2. Apothecia (× 10)
3. Section of Apothecium (*a*) and Pycnidium (*b*) (× 100)

AMIDE SYNTHESIS IN PLANTS

IV. Aspartase in Germinating Seedlings

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Received December 16, 1947

Since the discovery by Quastel and Woolf (1926) of the enzyme aspartase which converts aspartic acid into fumaric acid and ammonia its activity in several micro-organisms has been studied. Virtanen and Tarnanen (1932) prepared the enzyme from *Bacterium fluorescens liquifaciens* while Gale (1938) was able to show its presence in *B. coli.* According to Hæhn and Leopold (1937) autolysates of bottom yeast are also capable of converting aspartic acid to fumaric acid and ammonia. The only observation on the occurrence of this enzyme in plants was made by Virtanen and Tarnanen (1932) who found it to be present in pea seedlings and leaves of young red clover. The significance of aspartase in the reactions involved in amide synthesis in plants is obvious. The formation of aspartic acid from succinic acid can take place by one of two routes—either by the amination of oxal-acetic acid formed from succinic acid *via* fumaric and malic acids or by the amination of fumaric acid itself by the synthetic action of the enzyme aspartase. Investigations were therefore carried out to ascertain the presence of this enzyme in seedlings of *Phaseolus mungo* and *Cicer arietinum*, previously studied in this laboratory from the point of view of amide synthesis, as well as in *Pisum sativum* which was investigated by Virtanen and Tarnanen.

Experimental

In germinating the seeds special precautions were taken to ensure sterility in view of the strong aspartase activity of bacteria. The seeds were first soaked in 95% alcohol for three minutes, washed in water and then placed in 0·2% mercuric chloride solution for a further period of three minutes. They were then washed thoroughly with water, soaked in water for twenty-four hours and germinated on sterile sand in large vessels. For determination of enzyme activity a uniform suspension of the finely macerated seedlings was used; after removal of the testa the seeds or seedlings

were washed with water, dried lightly on filter-paper and then ground thoroughly in a cooled granite mortar. The ground brei after being dispersed in sufficient water to make 10 ml. of suspension for 1 gm. of fresh material was allowed to autolyse under toluene for twenty-four hours at 37° before use. The *l*-aspartic acid which was used as substrate was prepared by hydrolysis of asparagine with 3N hydrochloric acid for three hours. The solution was neutralised with ammonia and the aspartic acid precipitated was washed free from chloride with ice-water. It was recrystallised from hot water and its purity established by determination of the nitrogen content.

The experimental solutions for studying enzyme activity were made up as follows, the aspartic acid used being first neutralised to sodium aspartate before being added to the enzyme.

Enzyme suspension	20 ml.
Aspartic acid	0·4 gm.
M/15 Phosphate buffer (pH 7)	10 ml.
Total volume	50 ml.

Toluene was used as antiseptic and the solutions kept at 37° during the experimental period. In several experiments sodium fluoride was added to a concentration of 0·5% to exclude the possibility of bacterial action. A control was run without the addition of aspartate.

Enzyme activity was determined by periodical estimations of ammonia. 10 ml. of the experimental solution was introduced into a 50 ml. centrifuge tube containing 2·5 ml. of 20% trichloracetic acid. The solution was then centrifuged for five minutes and the centrifugate filtered through paper into a 25 ml. standard flask. The residue in the centrifuge tube was washed once with 10 ml. of 2·5% trichloracetic acid and the washings after centrifuging and filtration combined with the first filtrate. The solution was now brought to pH 7 and the ammonia determined according to the method of Pucher *et al.* (1935) by distilling *in vacuo* at 40° in Parnas and Klisiecki apparatus. The ammonia liberated was absorbed in sulphuric acid, nesslerised and determined colorimetrically. A similar estimation was simultaneously made on an aliquot of the control solution containing no aspartic acid.

Results

In the following tables ammonia is expressed in mg. per 10 ml. of the reaction mixture.

TABLE I

Phaseolus mungo, 2-*day old seedlings*

(*a*) without sodium fluoride (*b*) with sodium fluoride

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0 ..	a. 0·102	0·11	..	..	0·008
	b. 0·102	0·102	..	..	..
48 ..	a. 0·141	0·216	0·039	0·106	0·067
	b. 0·141	0·196	0·039	0·094	0·056
96 ..	a. 0·163	0·253	0·061	0·143	0·082
	b. 0·153	0·230	0·051	0·130	0·079
168 ..	a. 0·163	0·398	0·061	0·288	0·227
	b. 0·153	0·358	0·051	0·256	0·205

TABLE II

Phaseolus mungo, 4 *day old seedlings*

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0 ..	a. 0·238	0·240	..	..	0·002
	b. 0·240	0·240	..	..	..
48 ..	a. 0·422	0·467	0·184	0·227	0·043
	b. 0·427	0·467	0·187	0·227	0·040
96 ..	a. 0·495	0·565	0·257	0·325	0·068
	b. 0·498	0·563	0·258	0·323	0·065
168 ..	a. 0·495	0·616	0·257	0·376	0·119
	b. 0·498	0·600	0·258	0·360	0·102

TABLE III

Phaseolus mungo, 6-*day old seedlings*

Time (Hours)	Ammonia gm.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0 ..	0·232	0·232	..	..	..
48 ..	Not determined				
96 ..	0·512	0·550	0·280	0·318	0·038
168 ..	0·512	0·584	0·280	0·352	0·072

TABLE IV

Cicer arietinum, 3-*day old seedlings*

(*a*) without sodium fluoride (*b*) with sodium fluoride

Time (Hours)		Ammonia mg.				
		Control	Experimental	Increase		Difference
				Control	Experimental	
0	..	a. 0·262	0·266	..	..	0·004
		b. 0·260	0·260	..	..	..
96	..	a. 0·380	0·456	0·118	0·190	0·072
		b. 0·378	0·450	0·118	0·190	0·072
168	..	a. 0·380	0·558	0·118	0·292	0·174
		b. 0·378	0·552	0·118	0·292	0·174

TABLE V

Cicer arietinum, 5-*day old seedlings*

Time (Hours)		Ammonia mg.				
		Control	Experimental	Increase		Difference
				Control	Experimental	
0	..	0·389	0·390	..	..	0·001
48	..	0·451	0·475	0·062	0·085	0·023
96	..	0·535	0·628	0·146	0·238	0·092
168	..	0·535	0·793	0·146	0·403	0·257

TABLE VI

Cicer arietinum, 7-*day old seedlings*

Time (Hours)		Ammonia mg.				
		Control	Experimental	Increase		Difference
				Control	Experimental	
0	..	0·420	0·420	..	..	..
48	..	0·496	0·515	0·076	0·095	0·019
96	..	0·612	0·696	0·192	0·276	0·084
168	..	0·615	0·811	0·195	0·391	0·196

TABLE VII

Cicer arietinum, 11-*day old seedlings*

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0 ..	0·463	0·463	..	..	..
48 ..	0·510	0·520	0·047	0·057	0·010
96 ..	0·542	0·587	0·079	0·124	0·045
168 ..	0·542	0·642	0·079	0·179	0·100

TABLE VIII

Pisum sativum, 3-*day old seedlings*

(*a*) without sodium fluoride (*b*) with sodium fluoride

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0 ..	a. 0·175	0·175	..	..	..
	b. 0·175	0·175	..	..	..
48 ..	a. 0·212	0·263	0·037	0·088	0·051
	b. 0·209	0·260	0·034	0·085	0·051
96 ..	a. 0·297	0·407	0·122	0·232	0·110
	b. 0·297	0·399	0·122	0·224	0·102
168 ..	a. 0·300	0·492	0·125	0·317	0·192
	b. 0·300	0·492	0·125	0·317	0·192

TABLE IX

Pisum sativum, 5-*day old seedlings*

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0 ..	0·252	0·255	..	..	0·003
48 ..	0·376	0·451	0·124	0·196	0·072
96 ..	0·499	0·648	0·247	0·393	0·146
168 ..	0·500	0·720	0·248	0·465	0·217

TABLE X

Pisum sativum, *7-day old seedlings*

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0 ..	0·387	0·287	..	..	..
48 ..	0·514	0·584	0·127	0·197	0·070
96 ..	0·605	0·770	0·218	0·283	0·165
168 ..	0·605	0·897	0·218	0·510	0·292

TABLE XI

Pisum sativum, *11-day old seedlings*

Time (Hours)	Ammonia mg.				
	Control	Experimental	Increase		Difference
			Control	Experimental	
0 ..	0·465	0·465	..	..	..
48 ..	0·682	0·738	0·217	0·273	0·056
96 ..	0·736	0·848	0·271	0·383	0·112
168 ..	0·740	0·935	0·275	0·470	0·195

DISCUSSION

It is clear from the results obtained that aspartase is present in the three seedlings examined. The possibility that the decomposition of aspartic acid in these experiments was brought about by bacterial contamination is definitely ruled out as the activity was unaffected by the presence of fluoride and toluene.

The observed activity was, however, not very strong being of the same order as was observed by Virtanen and Tarnanen; the amount of aspartic acid decomposed at the time of maximum enzyme activity was between 4 and 5 mg. per gm. of seedling material during the experimental period of 168 hours. Further the activity of the enzyme diminished with the growth of the seedlings being maximum in *Phaseolus mungo* on the second day, in *Cicer arietinum* on the fifth day and in *Pisum sativum* on the seventh day after germination. The explanation for the low concentration of aspartase may be that the function of the enzyme in plants is a synthetic one and that its activity is therefore dependent upon the integrity of the cell structure; or

alternatively that the main path of aspartic acid synthesis in plants is *via* oxalacetic acid—the importance of which in carbohydrate metabolism is indicated by several lines of evidence—and that aspartase plays only a secondary role in the synthesis of aspartic acid from succinic acid.

Summary

The presence of the enzyme aspartase which converts aspartic acid into fumaric acid and ammonia has been demonstrated in seedlings of *Phaseolus mungo*, *Cicer arietinum* and *Pisum sativum*. Its significance in aspartic acid synthesis in plants is briefly discussed.

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ERRATUM

Vol. XXVII, Sec. B, No. 1

Plate I, relating to the paper entitled "Anatomy of Sugarcane Stalk as Influenced by Top Borer (*Scirphophaga nivella* F.) Attack," should be between pp. 2 & 3, *instead of* between pp. 16 & 17, as now inserted.

STUDIES IN THE GENUS PHYTOPHTHORA—II

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Received January 2, 1948

(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

In the previous article Thomas *et al.* (1947) discussed the behaviour of different isolates of *Phytophthora palmivora* in paired cultures and based on the results of their studies, all the isolates studied were brought under the same species, *viz.*, *P. palmivora* Butl. though they were being previously referred to three or more different species. Further investigations were continued with these and some more isolates belonging to *P. parasitica* and *P. colocasiæ*. The results of these investigations are described in this paper.

Materials and Methods

As mentioned in the earlier communication (Thomas *et al.*, 1947) the isolates of *Phytophthora* already available in stock collection in the section were utilised. New isolates were brought into culture from infected castor and agave leaves when infection by the fungus occurred in nature on the Central Agricultural Research Station, Coimbatore. Isolates from bread-fruit were obtained from diseased fruits got down from South Kanara. Authentic type cultures of *P. parasitica* and *P. colocasiæ* were received from the Indian Agricultural Research Institute, New Delhi, through the courtesy of Mr. Dastur. Dr. Asthana was kind enough to send us from Nagpur the culture of *P. parasitica* var. *piperina*. The paired cultures were grown on oat agar in petri-dishes or test-tubes in the months of October to January when the laboratory temperature was in the neighbourhood of 26° C.

1. *Isolate from castor* (Ricinus communis)

Castor plants growing in the experimental plots of the Oilseeds Specialist at Coimbatore were found infected during the north-east monsoon in October 1946, by a leaf-blight caused by *Phytophthora*. The fungus was isolated and brought into pure culture from single hyphal tips. A white luxuriant aerial growth developed on oat agar. Numerous ob-pyriform sporangia and intercalary hyaline to yellowish brown chlamydospores were formed. But sexual bodies did not develop even after four months.

This isolate was grown in paired cultures with other isolates and oospores were formed in the course of one week as shown in Table I.

TABLE I

Statement showing the result of growing this isolate in paired cultures

Paired with isolate from			Result			
Arecanut	*P. palmivora*	plus strain ..	Oospores formed in	6	days	
Clerodendron	do	do ..	do	do	5	do
Agave	do	minus strain ..	do	do	5	do
Hevea	do	do ..	do	do	5	do
P. parasitica	type culture	..	do	do	4	do
P. colocasiæ		..	do	do	5	do

Oospores were formed with both the plus and minus strains of *P. palmivora* and also with *P. parasitica* and *P. colocasiæ*. In single strain cultures however oospores are not developed either by this isolate or by the others with which it was paired. *P. parasitica* has been recorded on castor and the isolate under study will normally be identified as *P. parasitica* from the sporangial and chlamydospore characters though it does not form oospores in single strain cultures. But the ease with which it pairs with the plus and minus strains of *P. palmivora* raises some important doubts about the taxonomic relationship of the former species. This is discussed later in detail.

2. *Isolate from* Agave wightii

The leaf-blight on this host caused by *Phytophthora* was prevalent at Coimbatore in the months of November and December 1946. The isolate was brought into pure culture by tissue culture which was further purified by transferring single hyphal tips. On oat agar a luxuriant white aerial growth which filled the tube was formed. Profuse formation of sporangia and chlamydospores took place. No sexual bodies developed even after four months.

Paired cultures were grown using this isolate as one of the strains and one or another isolate of known performance. The results are given in Table II.

The behaviour of this isolate is identical with that of the old isolate from the same host already available in the section (Thomas *et al.*, 1947) in the formation of oospores with strains of *P. palmivora*. But the old isolate is reported (Marudarajan, 1941) to have produced oospores in single strain cultures for some time after isolation though this capacity was lost after two years' continuous culturing on agar media. The new isolate however does not form oospores in single strain cultures even after three months. The isolate readily combines with *P. parasitica* and *P. colocasiæ* also and forms oospores in paired cultures with these species.

TABLE II

Statement showing the results of growing the isolate from Agave wightii *in paired culture*

Paired with isolate from	Results
Clerodendron *P. palmivora* plus ..	Oospores formed in 5 days
Arecanut do do ..	do do 5 do
Hevea do minus ..	No oospores formed
Spondias do do ..	do
Castor *P. parasitica* ..	Oospores formed in 3 days
P. parasitica type culture ..	do do 5 do
P. parasitica var. *piperina* ..	do do 5 do
P. colocasiæ type culture ..	do do 5 do

3. *Isolate from Breadfruit* (Artocarpus incisa)

The performance of two earlier isolates from this host have been described in an earlier communication (Thomas *et al.*, 1947). This isolate was obtained from infected fruits of *Artocarpus incisa* brought from South Kanara. Its growth on oat agar was less profuse than the isolates from castor and agave. Ob-pyriform sporangia which are usually terminal and hyaline, and yellowish thick-walled spherical or subglobose intercalary chlamydospores are formed in large numbers. Sexual reproduction is not evident for the first ten days of growth. But numerous oogonia and oospores are formed in 15 days in single strain cultures. The culture was isolated from single hyphal tips and therefore this isolate is found to be homothallic and fertile. The oogonium is hyaline or light yellow and persistent. The antheridium is amphigynous. The oospore is thick walled and yellowish brown. The relative measurements of oogonia and oospores were 28·6 (20–31) μ and 27·5 (18–29) μ respectively. These are not far different from the average of the measurements obtained by Marudarajan (Thomas, 1941) for the sexual bodies of an isolate from breadfruit, *viz.*, oogonia 30·5 μ (19·3–38·5) μ and oospores 28·3 (17·5–35) μ.

This isolate from breadfruit was also grown in paired cultures with other isolates whose sexual behaviour was already known and the influence on the rapidity of oospore formation was observed.

From the results below it is found that the isolate from breadfruit forms oospores in single strain cultures in 11 days. But when paired with the plus strain of *P. palmivora* or with *P. parasitica* or *P. colocasiæ* sexual bodies begin to develop within thirty-six hours to 3 days after inoculation. On the other hand, when it is paired with the minus strains of *P. palmivora* oospore formation is visible only after ten days. In these the oospores

TABLE III

Statement showing the effect of pairing the isolate from breadfruit with other isolates of Phytophthora

Paired with isolate from			Results
Jack	*P. palmivora*	plus strain ..	Oospores formed in 48 hours
Betel vine	do	do ..	do do 48 do
Citrus	do	do ..	do do 48 do
Colocasia	do	do ..	do do 48 do
Areca	do	do ..	do do 48 do
Clerodendron	do	do ..	do do 36 do
Spondias	do	minus strain ..	No oospores upto 10 days
Hevea	do	do ..	do do
P. parasitica		type culture ..	Oospores formed in 3 do
P. colocasiæ		do ..	do do 3 do
Isolate from breadfruit (single strain)		..	do do 11 do

might have been formed by the breadfruit isolate itself and not by the pairing of the two strains. Thomas *et al.* (1947) have found that the first isolate of *P. palmivora* from breadfruit behaved as a minus strain. It formed oospores in single strain cultures when first isolated by Marudarajan (Thomas, 1941) but this capacity had been lost in later years, after having been grown on agar media for over four years. Thus a homothallic fertile strain has apparently changed later on into a minus strain. The present isolate also gives an indication of its probable future change. At present it is homothallic and fertile but it pairs easily with plus strains of *P. palmivora* or with homothallic non-fertile strains of *P. parasitica* or *P. colocasiæ* while there is no evidence of combination with the minus strains, thus exhibiting a tendency towards the development of the 'minus' character.

Gadd (1927) obtained an isolate from breadfruit which behaved as a member of his 'rubber' group (which is equivalent to the minus strain of *P. palmivora*). Apparently breadfruit is parasitized by both homothallic (fertile) and minus strains of *P. palmivora* in different localities. But eventually even the homothallic strain changes into the minus strain when grown on agar media for a length of time.

4. P. parasitica *Dast. var.* piperina *Dast. on* Piper betle

A type culture of this isolate was kindly supplied by Dr. Asthana, the Mycologist to the Government of Central Provinces and Berar, Nagpur. It was found to grow luxuriantly on oat agar forming a white dense aerial growth. Dastur (1935) had described this as a new variety of *P. parasitica*. His isolates were producing numerous oospores on agar media. But the culture received from Nagpur formed sporangia and chlamydospores but

not sexual bodies. It was however grown in paired cultures with other isolates of known reaction and the results are given below.

TABLE IV

Statement showing the formation of oospores in paired cultures

Paired with the isolate from			Results
Aecanut (Tyagli)	*P. palmivora*	minus strain ..	Many oospores in 8 days
Coconut	do	do ..	Less number in 6 do (Oogonia deep coloured thickened)
Hevea	do	do ..	Numerous oospores in 4 days
Jatropha	do	do ..	do do 5 do
Agave	do	do ..	do do 4 do
Spondias	do	do .	Less number do 4 do
Tomato	do	plus strain ..	do do 8 do
Areca	do	do ..	do do 8 do
P. parasitica		..	Numerous oospores 4 do
P. colocasiæ		..	do do 7 do

This isolate combines with both the plus and minus strains of *P. palmivora*, with *P. parasitica* and *P. colocasiæ*. In this respect it is akin to the isolate from castor. The intensity of oospore formation varies in the different combinations. Oospores are formed in greater abundance with isolates recently brought into culture than with older ones.

Dastur (1935) raised this isolate to a new variety of *P. parasitica*, the main reason being the larger sizes of the oogonium and the oospore compared to those of *P. parasitica* though he acknowledges on the same page that " the size of the oogonium is not constant and is influenced by the medium ". In later isolations of *P. parasitica* he had found bigger oogonia and oospores. In authentic cultures of *P. parasitica*, Tucker (1931) has found that oospores are very variable in size and exhibit a range in diameter from 12 to 35 μ. In the light of the above observations it becomes evident that this isolate is not different from the type of *P. parasitica* and there is not enough justification to classify it as a new variety.

The sexual bodies formed in some of the paired cultures were measured and the measurements are given below together with the sizes recorded by Dastur.

These measurements fall within the range of those recorded from paired cultures of *P. palmivora* strains (Thomas *et al.*, 1947).

TABLE V

Statement showing the size of sexual bodies in paired cultures

Particulars	Oogonia		Oospores	
	Mean μ	Range μ	Mean μ	Range μ
P. parasitica var *piperina* Dastur's measurements	38·4	20·4–40·8	26·1	17·8–33·1
do + *P. palmivora* (Tyagli)		24 –32		22 –27
do + do Tomato		24 –33		21 –30
do + do Cocoanut	31·5	26 –43	26	20 –31
do + *P. parasitica* ..	28·0	21 –34	23	16 –25
do + *P. colocasiæ* ..	29·1	23 –33	22·5	18 –26

5. P. colocasiæ*i Rac.*

A type culture of this fungus was obtained from the Indian Agricultural Research Institute, New Delhi, through the courtesy of the Head of the Division of Mycology. Its growth on oat-agar is not so luxuriant as that of the isolates from betelvine or arecanut. The cultures are in need of frequent transfers to fresh agar media in order to keep them going and to prevent them from dying out.

Raciborski (1900) was the first to describe this fungus from Java. Butler and Kulkarni (1913) conducted a detailed study of the same fungus from India. Their isolates produced sporangia, chlamydospores and oospores. The cultures now received from New Delhi were of the non-oospore forming type (non-fertile) and produced only sporangia and chlamydospores in single strain culture. The behaviour of this isolate when

TABLE VI

Statement showing the development of oospores in paired culture

Paired with	Results
P. palmivora (Tomato) plus strain ..	Oospores formed in limited numbers in 7 days
do (Jack) do ..	do do 8 do
do (Betelvine) do ..	Few oospores in 9 days
do (Coconut) minus strain ..	Numerous oospores in 5 days
do (Hevea) do ..	do 4 do
do (Breadfruit) do ..	do 3 do
do (Agave) do ..	do 4 do
do (Jatropha) do ..	do 4 do
do (Spondias) do ..	do 4 do
do (Areca-tyagli) do ..	do 9 do
P. parasitica ..	do 4 do
do var. *piperina* ..	do 6 do

grown in paired cultures with other isolates of *P. palmivora* and *P. parasitica* was studied and the results are shown above.

The behaviour of this isolate is akin to that of *P. parasitica* var. *piperina*. More oospores develop in combination with the minus strains of *P. palmivora* than with the plus strains. The studies of this fungus have shown that it closely resembles *P. parasitica* and *P. palmivora* in its morphological characters. The ease with which it pairs with these two species and forms oospores of the same type brings out the affinities between them more clearly.

The size of the sexual bodies observed by Butler and Kulkarni (1913) for *P. colocasiæ* agrees closely with those of oogonia and oospores formed in paired cultures. These are given below.

TABLE VII

Statement showing the sizes of oogonia and oospores

Combination	Oogonia		Oospores	
	Mean μ	Range μ	Mean μ	Range μ
P. colocasiæ (Butler and Kulkarni)	29·5	24–31	23	20 –28
P. colocasiæ + *P. palmivora* (tomato)	26	24–28	21	18·5–22
do + do (areca-tyagli	31·5	25–41	24	18 –34
do + do (Spondias)	27·5	21–34	22	15·5–25
do + *P. parasitica*	28	24–31	22	18 –25
do + *P. parasitica* var. *piperina*	29·1	23–33	22·5	16 –25

The measurements are comparable to those recorded for sexual bodies formed in paired cultures of *P. palmivora*. In this isolate one can however detect an indication of the weakening of the homothallic tendency since it forms larger number of oospores with the minus strains of *P. palmivora* than with the plus strains. Probably this isolate will in course of time lose its capacity to combine with the plus strains and itself may behave as a plus strain. It may be interesting to note the behaviour of an isolate of *P. palmivora* from *Colocasia antiquorum* recorded by Thomas *et al.* (1947) which had the sporangial character usually attributed to *P. colocasiæ* but was non-oospore forming and behaved as a plus strain combining with the minus strains of *P. palmivora*. All these factors indicate the necessity for merging this species with *P. palmivora*. Leonian (1925) was the first to express a similar view.

6. Phytophthora parasitica *Dast.*

Mr. Dastur, the Head of the Division of Mycology, Indian Agricultural Research Institute, New Delhi, kindly sent us an authentic type culture of *P. parasitica* for our studies. This species was first described by Dastur (1913) from India having been isolated from blighted leaves of *Ricinus communis*. Since then it has been studied by numerous workers in different parts of the world on various hosts. The original isolate has been described as forming oospores in single strain cultures. But Tucker (1931) found that the " occurrence of oogonia and oospores in cultures is uncertain. They may appear only after several months or frequently not at all." The isolate obtained from New Delhi did not produce oospores at laboratory temperature, *i.e.*, 24°–30° C. even after six months.

Paired cultures in combination with other isolates of *P. palmivora*, *P. colocasiæ* and *P. parasitica* var. *piperina* were grown and the results are recorded below.

TABLE VIII

Statement showing the development of oospores in paired cultures

Paired with isolate	Results
P. palmivora Tomato plus strain ..	Oospores numerous in 7 days
do *Colocasia* do ..	do formed in 8 do
do Jack do ..	do do 5 do
do Betelvine do ..	do do 10 do
do Citrus 1 do ..	do do 10 do
do Areca do ..	do do 10 do
do *Clerodendron* do (new) ..	do numerous in 4 do
do Coconut minus strain ..	do formed in 10 do
do Agave do (new) ..	do numerous in 4 do
do *Spondias* do (new) ..	do do 4 do
do *Hevea* do ..	do do 4 do
do Breadfruit do (new) ..	do do 4 do
P. colocasiæ ..	do many 10 do
P. parasitica var. *piperina* ..	do do 4 do

The isolate combines readily with heterothallic and homothallic isolates of *Phytophthora*. It forms oospores with fertile as well as non fertile homothallic strains. But when grown in single strain cultures oospores are not formed. Further, when this isolate is paired with an isolate of *P. palmivora* from *Spondias mangifera* which had become neutral no oospores developed even after a month. This demonstrates the fact that oospore formation in paired cultures is governed by the biological nature of the isolates that are brought together and the neutral strains do no pair at all.

The size of the sexual bodies produced by this isolate in paired culture falls within the range recorded for *P. palmivora* or *P. parasitica* as shown below.

TABLE IX

Measurements of sexual bodies produced by P. parasitica *with other isolates of* Phytophthora

Particulars	Oogonia		Oospores	
	Mean μ	Range μ	Mean μ	Range μ
P. parasitica + *P. palmivora* (tomato) ..	26	21–31	20	15–22
do do (citrus 1) ..	27	24–34	21	15–28
do do (*Jatropha*) ..	29	24–37	22	18–29
do do (Coconut) ..	28	25–32	22	21–25
do *P. colocasiæ* ..	28	24–31	22	18–20
do *P. parasitica* var. *piperina* ..	28	21–34	23	16–25

The range of measurements of the oogonia of *P. parasitica* on agar media is 15–35 μ and of oospores 13–26 μ. In some strains the oospores "which mostly fill the oogonial cavity range from 12 μ to over 35 μ" (Tucker, 1931). The sizes of oogonia and oospores formed in homothallic or paired cultures of *P. palmivora* range from 16 to 39 μ and 12 to 28 μ respectively.

The two allegedly different species have almost identical ranges of size of sexual bodies and the oospores formed in the paired cultures included in these studies also fall within this range.

DISCUSSION

The results of the studies detailed above show the necessity for the revision of the current taxonomic classification of these isolates. The different isolates studied are at present grouped into *P. colocasiæ*, *P. palmivora*, *P. parasitica* and *P. parasitica* var. *piperina*. The criteria influencing the determination of species of *Phytophthora* include the nature of growth on culture media, temperature relations, size and shape of sporangia and chlamydospores, the readiness with which sexual bodies are formed, their size, the nature of the antheridia and pathogenicity of the isolates. We shall consider these factors with reference to the isolates under study and see how far they can be relied upon.

The nature of growth of different isolates on agar media exhibit certain differences. But these differences cannot be relied upon for specific differentiation. Some isolates of *P. parasitica* and *P. palmivora* exhibit almost

identical growths of aerial mycelium on oat agar and could not be distinguished from one another. Leonian (1925) has also found that his observations afforded no justification for the practice of making the production of aerial hyphæ a distinctly specific character. Numerous intergradations in the amount of aerial hyphæ may be produced by the strains of a single species. Hence this character cannot be relied upon. Tucker (1931) also found that the same single strain culture of *P. parasitica* may produce various types of growth on different media.

The same author has stated that the main difference between *P. parasitica* and *P. palmivora* is in their temperature relationship. The same differential behaviour is cited between *P. colocasiæ* and *P. parasitica* also. He found that *P. parasitica* grew on cornmeal agar at 35° C. while *P. palmivora* and *P. colocasiæ* did not. He further states that " the use of the ability to grow at certain temperatures as a criterion for the identification of species seems to be justified." Leonian (1934) in his studies on temperature relationships of many species of *Phytophthora* found that in both *P. parasitica* and *P. palmivora* some isolates grew at 35° C. while others did not. Mehrlich (1936) found that temperature relations for separating *P. parasitica* and *P. palmivora* do not hold good for isolates of *P. parasitica* from heart-rot of pine-apples. Six isolates of *P. parasitica* did not grow at 35° C. while four others did and all of them were morphologically alike.

In order to verify the behaviour of the local isolates one type culture of each of *P. colocasiæ*, *P. palmivora* and *P. parasitica* was inoculated in triplicate on oat agar in petri-dishes and kept in an incubator at 35° C.$\pm\frac{1}{2}$° C. When examined on the fifth day, all the three exhibited growth, the largest diameter being in *P. parasitica* and next in *P. palmivora* and still less in *P. colocasiæ*. Though there were differences in the amount of growth all of them had grown. Thus the ability to grow at 35° C. is exhibited by all the three isolates. Hence this reaction cannot be utilised for taxonomic purposes. Further the isolates under study are all tropical organisms and 35° C. is a temperature to which they will be exposed during certain parts of the year and consequently they must have become acclimatised to such high temperatures. The culture of the local isolate from castor can be kept alive for a long period. But the type culture of *P. parasitica* from Delhi died out in a few months under local conditions. It is, however, shown that the differences observed by Tucker between these three species does not always hold good.

Sporangial characters have been overemphasized by a number of mycologists in differentiating species of *Phytophthora*. *P. colocasiæ* is said to be

easily distinguished from other allied species by its elongated narrowly ovate sporangia which are shed with remnants of the pedicel attached to the base of the sporangium. When cultures of *P. colocasiæ* are examined these features are not found to be constant. Broadly oval sporangia as in *P. parasitica* or *P. palmivora* are also formed. Further some isolates of *P. palmivora* develop elongated sporangia resembling those of *P. cclocasiæ* (Leonian, 1936; Thomas *et al.*, 1947). The size and shape of the sporangia in these three species are so variable and unstable that an undue emphasis should not be laid on these characters for specific differentiation. There are no constant differences between the sporangia of the three species.

The same may be said about the chlamydospores. These bodies are produced in varying numbers by the different isolates of these three species and no specific difference could be made out between them.

Considering the development of the oogonia and oospores it has been found that all the three species have been known to form these bodies in single strain cultures and no differences exist between them regarding the size attained by these bodies. The antheridium in all the three species is amphigynous. *P. colocasiæ* and *P. parasitica* were distinguished from *P. palmivora* by the readiness with which oospores were formed in single strain cultures of the two former species. Some isolates of *P. meadii* which is now considered as *P. palmivora* formed oospores readily. The studies recorded above have further shown that depending on the isolates all the three species are capable of forming oospores in single strain cultures. It has also been found that in all the three species non-oospore forming cultures are available. The isolates are non-oospore forming either soon after fresh isolation or after some generations on agar media. Other workers have also found (Tucker, 1931) that the occurrence of oogonia and oospores in culture in *P. parasitica* is uncertain. " Seldom do they appear promptly and frequently not at all." Thus this character cannot be relied upon and no difference is seen between the three species in the size or shape of oogonia, oospores or antheridia, when these are developed.

Pathogenicity has been given an undue importance in delimiting species. Though this character may be of value in obligate parasites, when dealing with organisms, like species of *Phytophthora* this method of differentiation is almost of no value for distinguishing species. *P. colocasiæ* was thought to be confined to *Colocasia antiquorium* and this phenomenon was made much of. But Thompson (1929) and Thet Su (1938) have found *P. colocasiæ* on *Piper betle.* Thomas *et al.* (1947) have isolated *P. palmivora* from *Colocasia antiquorum.* A consideration of the host range of *P. palmivora* and

P. parasitica in nature reveals that both are formed on most of the recorded host plants. Below are given some of the recorded hosts of the three species.

TABLE X

Statement showing some of the common recorded hosts of P. palmivora, P. colocasiæ *and* P. parasitica

Host	Country where recorded	Identification of pathogen	Parts affected	Isolated by
1. *Citrus* spp. ..	Bombay	*P. palmivora*	Foot rot and fruit rot	Uppal
	Madras	do	Fruit rot	Thomas *et al.*
	Philippines	*P. parasitica*		Reinking
	Porto Rico	do	do	Dreschter
2. *Cocos nucifera* ..	Madras	*P. palmivora*	Bud rot	Shaw and Sundararaman
	Philippines	*P. parasitica*	do	Reinking
	Porto Rico	*P. palmivora*	do	Tucker
3. *Colocasia antiquorum*	India	*P. colocasiæ*	Leaf spot and Blight	Butler and Kulkarni
	Madras	*P. palmivora*	do	Thomas *et al.*
	Java	*P. colocasiæ*	do	Raciborski
4. *Gossypium barbadense*	Porto Rico	*P. parasitica*	Boll rot	Tucker
	St. Vincent	*P. palmivora*	do	Ashby
	Montserrat	do	do	Wakefield
5. *Lycopersicum esculentum*	New York	*P. parasitica*	Fruit rot	Reddick
	U.S.A.	do	do	Lavelleé
	Madras	*P. palmivora*	do	Ramakrishna and Sowmini
6. *Piper betle* ..	Central Provinces (India)	*P. parasitica* var. *piperina*	Wilt	Dastur
	Bengal	*P. palmivora*	do	McRae
	Madras	do	do	Thomas *et al.*
	Malaya	*P. colocasiæ*	do	Thompson
	Burma	do	do	Thet Su
7. *Solanum melongena*	Philippines	*P. palmivora*		Reinking
	do	*P. parasitica*	Fruit rot	Ocfemia
	do	do	do	Reinking
8. *Theobroma cacao*	Java	*P. palmivora*	Pod rot	Ashby
	Ceylon	do	do	Gadd
	Surinam	*P. parasitica*	do	Stahel

The above list includes instances where the pathogen isolated from the same host affected by similar diseases has been differently named. The frequency with which *P. palmivora* and *P. parasitica* have been recorded on the same host plants denotes the identical pathogenic qualities of those two. It is surmised that the relative identification of the pathogen might have been influenced by the development of sexual bodies in cultures or their

absence. *P. colocasiæ* is however restricted in its parasitism. For a long time it was known only on *Colocasia antiquorium* but in Malaya Thompson (1929) and in Burma Thet Su (1930) have recorded it on *Piper betle* also. Thomas *et al.* (1947) have isolated *P. palmivora* from leaves of *C. antiquorum.* Restricted parasitism of certain strains of the same species is well known in fungi. It is also known that the pathogenic ability of facultative suprophytes can be changed by slow ' education ' of the particular strains. Therefore the exclusive use of pathogenicity to distinguish species among facultative saprophytes is not a reliable guide.

The close relationship between these species is further illustrated by the ease with which one is mistaken for the other. Thompson (1929) concluded from his studies of a number of isolates of *Phytophthora* that *P. parasitica* may develop homothallic and heterothallic strains and the latter can be regarded as being atypical members of *P. palmivora.* The tobacco fungus was originally described by Breda de Haan in 1896 as *P. nicotianæ.* Tisdale (1922) observed the close similarity between this and *P. parasitica* which was confirmed by Leonian (1925). Lester Smith (1927), however, observed that the size of the sporangium in *P. parasitica* agreed with that of *P. nicotianæ* but considered the latter to be a strain or form of *P. palmivora* from its behaviour in paired cultures with isolates of that species. Ashby (1928) included *P. nicotianæ* in *P. parasitica.* Tucker (1931) wanted to give a distinguishing name to the organism responsible for black shank of tobacco and named it *P. parasitica* var. *nicotianæ.* Thomas *et al* (1947) found that the black shank organism could be classified as *P. palmivora* on account of its behaviour in paired cultures and the absence of any distinguishing characters to consider it as a separate species or variety. The frequent changes in the nomenclature of this fungus shows how the two species are very much alike and are liable to be interchanged according to the isolate under study.

The readiness with which the isolates at present classified as *P. colocasiæ*, *P. palmivora* and *P. parasitica* combine in paired cultures and form oospores is a further proof of their close specific affinity. Lenonian (1931) followed the behaviour of 85 cultures of *Phytophthora* belonging to his ' *Omnivora* group '. The formation of oospores in paired cultures by 48 of these is considered by him and rightly too as due to heterothallism and not hybridization between species. If we are to consider the pairing between *P. parasitica* and *P. palmivora* as one of hybridization as believed to be by Narasimhan (1930) there must be some features in the resulting oospores which distinguish them from those formed when two heterothallic isolates of *P. palmivora* are paired. Narasimhan took this view owing to the size

of the oospores formed when *P. parasitica* was paired with *P. palmivora* which he thought was intermediate between those of the oospores of the two species. But it is now known that the size of oospores obtained by Narasimhan in his paired cultures is even obtained in single strain cultures of *P. palmivora* or of *P. parasitica*. Therefore his reasoning that hybridization has taken place in his cultures is not tenable. The formation of cospores in paired cultures of the isolates included in these studies has to be considered as union between heterothallic forms of the same species and not as instances of hybridization between different species.

It may be mentioned here that ' plus ' and ' minus ' strains of *P. palmivora* were grown with *P. cambivora* and *P. cactorum*. Oospores did not develop in the cultures with the former species. In the cultures with the latter species oospores were formed but they were of the ' *Cactorum* ' species with paragynous antheridia in the majority of sexual bodies.

The results of these investigations point to the desirability of amalgamating *P. parasitica*, *P. palmivora*, *P. colocasiæ* and *P. parasitica* var. *piperina* into one species as there are no valid, reliable or constant differences between them to keep them separate. Leonian (1925) has already suggested the combination of all these under the binomial *P. omnivora*. This was objected to by Ashby (1928) who pointed out that the sporangia of *P. colocasiæ* are distinguishable from those of *P. parasitica* and the ready development of oospores separates it from *P. palmivora*. Later studies by various authors have shown that both these characters are not constant and are exhibited by the other species also. Another objection raised by Ashby (1928) is, in regard to the use of the binomial *P. omnivora*. The specific name ' *omnivora* ' was coined by De Bary without regard to the prior name of ' *cactorum* ' used to denote the same species (Fitzpatrick, 1930). Therefore the name *P. omnivora* becomes a synonymn of *P. cactorum* and cannot be revived to include the three species as intended by Leonian. Tucker (1931) has considered *P. omnivora* as a synonym of *P. cactorum*. In a later publication Leonian (1934) has suggested the merging of *P. parasitica* and *P. palmivora* under the latter species keeping *P. colocasiæ* as a separate species. Our studies support his original view that all the three species should be merged into one. The obvious question which arises is, which is the name to be retained for the species in which three old species are merged. The correct procedure according to the rules of nomenclature is to adopt the oldest name, *i.e.*, *P colocasiæ*. Dr. Bisby who was consulted about the specific name to be adopted held the view that if we were satisfied about the necessity for merging all the three species into one, the specific name ' *colocasiæ* ' ought to be retained as it is the oldest and sufficiently well known. Accepting

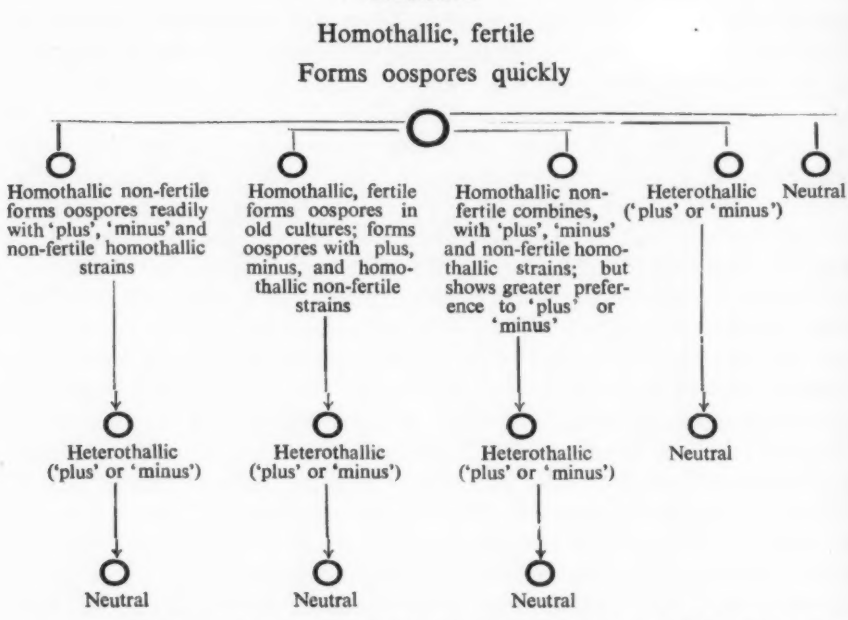
Dr. Bisby's view it is proposed to combine *P. colocasiæ*, *P. palmivora* and *P. parasitica* into one species *P. colocasiæ*.

The behaviour of the isolates of this emended species in single strain and paired cultures is variable. Some isolates are fertile and produce oogonia and oospores in single strain cultures quickly. Other isolates form these bodies very late. Both these may be described as homothallic and fertile. Still other isolates do not form oospores in single strains but combine readily with the plus and minus strains. These may be considered as homothallic but non-fertile and require the sexual stimulation of another biologically active isolate to form oospores. Two homothallic but non-fertile forms also are capable of combination as the isolates of *P. colocasiæ* and *P. parasitica* under study. Variations from this reaction are exhibted by some isolates which produce more oospores with the ' plus ' strain than with the ' minus ' strain or *vice versa*. Since neutral isolates do not form oospores in combination with homothallic non-fertile isolates the development of oospores must be considered to be governed by biological (sexual) nature of the isolate and not by the biochemical stimulation by the presence of any other isolate. Such homothallic non-fertile strains which exhibit a preference for the ' plus ' or ' minus ' strain might gradually give rise to the heterothallic strains of the ' minus ' or ' plus ' types themselves. With continued growth on agar media some of these become neutral, and do not form oospores with any combination of isolates. Thus from an original fertile homothallic isolate, non-fertile homothallic or heterothallic or neutral strains may be developed in course of time. This might represent the course of development of the sexual behaviour of this species in nature also with the result that different isolates behave in different ways according to their genetic make up. These changes can be attributed to new combinations, and segregations that occur during and after the sexual reproduction in *Phytophthora*. The germination of the oospore is usually accompanied by the meiotic division of the fertilised nucleus and this feature must account for the segregation of factors and formation of new races and strains. Mutations occurring during the life of the isolate in pure cultures lead to further variations.

Edgerton *et al.* (1944) have noticed during their studies on the genetics of *Glomerella* that perithecia-forming strains, ' plus ' strains and ' minus ' strains exist in the isolates of the fungus. Perethecia-forming strains may combine with ' plus ' or ' minus ' strains leading to the formation of larger number of perithecia. They have also seen that a ' plus ' strain may give rise to a ' minus ' strain or perithecia producing strain. Chilton and Wheeler (1947) are of opinion that new strains of *Glomerella* arise by mutation

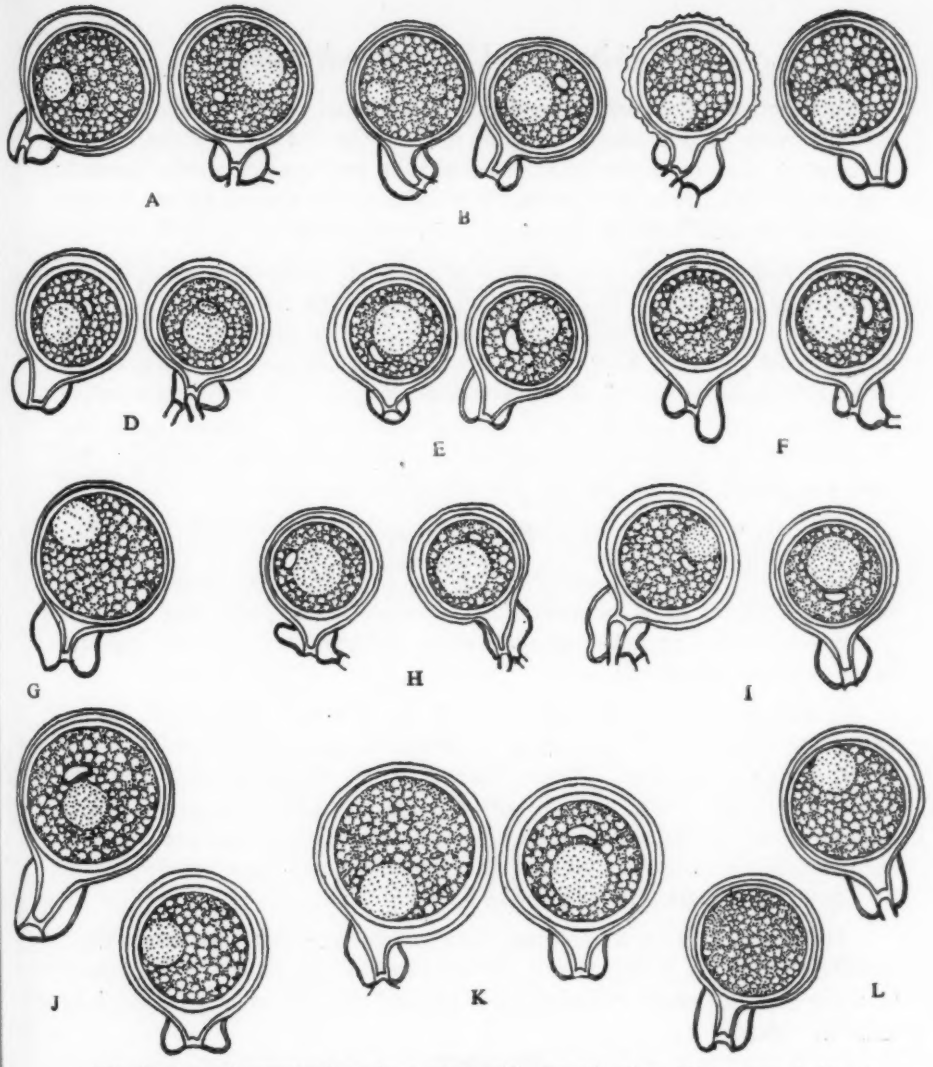
from the 'plus' strain. The above is comparable to what takes place in the cultures of *P. colocasiæ*.

Speculation on the origin of the different isolates studied is not attempted but a suggestion is put forward indicating the possible course of development of the different strains as shown below:


PARENT
Homothallic, fertile
Forms oospores quickly
Homothallic non-fertile forms oospores readily with 'plus', 'minus' and non-fertile homothallic strains
Homothallic, fertile forms oospores in old cultures; forms oospores with plus, minus, and homothallic non-fertile strains
Homothallic non-fertile combines, with 'plus', 'minus' and non-fertile homothallic strains; but shows greater preference to 'plus' or 'minus'
Heterothallic ('plus' or 'minus')
Neutral
Heterothallic ('plus' or 'minus')
Heterothallic ('plus' or 'minus')
Heterothallic ('plus' or 'minus')
Neutral
Neutral
Neutral
Neutral

Leonian (1934) has emended the description of *P. palmivora* (including *P. parasitica* and *P. palmivora*). With slight alternations the description for the emended species of *P. colocasiæ* (Racib.) Thom. and Ram. now proposed will read as follows:—

The emended species of *Phytophthora colocasiæ* (Racib.) Thom. and Ram. (merging *P. colocasiæ*, *P. palmivora*, *P. parasitica* and its varieties) enjoys a world-wide distribution and has been recorded on numerous host plants thus exhibiting an omnivorous habit. The growths of this fungus on agar media exhibit wide variation. Aerial hyphæ may be luxuriant, scanty or sometimes absent; sub-merged hyphæ, smooth or gnarled, even or uneven; the temperature tolerance varies according to the country of origin of the isolate, from 30° C. to 37·5° C. Sporangia distinctly papillate, greatly variable in size and shape; chlamydospores spherical



The drawings of oospores have been made with the aid of an Abbe camera lucida at uniform magnification of × 680.

Oospores formed in paired cultures of different combinations :—

A.	*P. palmivora* (plus, Tomato)	×	*P. parasitica* var. *piperina*
B.	do (minus, Areca)	×	*P. parasitica*
C.	do (minus, Coconut)	×	do var. *piperina*
D.	do (plus, Tomato)	×	*P. parasitica*
E.	*P. colocasiæ*	×	do
F.	*P. palmivora* (minus, *Jatropha*)	×	do
G.	do (minus, Coconut)	×	do
H.	do (plus, Citrus)	×	do
I.	do (plus, Betelvine)	×	do
J.	do (minus, Coconut)	×	*P. colocasiæ*
K.	do (minus, Areca)	×	do
L.	do (plus, Tomato)	×	do

or subspherical, light or deep coloured, terminal or intercalary; some isolates may produce more of these bodies than others. Sexual bodies present or absent: heterothallic, homothallic and neutral strains present; antheridia typically amphigynous; size of oogonia and oospores greatly variable, oogonia 13–41 μ, oospores 12–35 μ in diameter.

We wish to express our indebtedness to Dr. Asthana of Nagpur and Mr. J. F. Dastur, Head of the Division of Mycology, New Delhi, for kindly supplying type cultures of certain isolates. To Dr. Bisby of the Imperial Mycological Institute, Kew, we are thankful for the advice on the choice of specific name. Mr. M. S. Balakrishnan helped us in isolating some of the strains and in making the diagrams. Miss C. K. Soumini made the measurements of oogonia and oospores in some of the paired cultures. We offer our thnaks to them for the help rendered.

Summary

Isolates of *Phytophthora* from castor, *Agave*, and breadfruit and authentic cultures of *P. parasitica*, *P. parasitica* var. *piperina* and *P. colocasiæ* were studied in detail in single strain cultures and in paired cultures in different combinations.

It was found that the isolates from the different hosts under study easily combined with *P. parasitica*, *P. parasitica* var. *piperina* and *P. colocasiæ*, forming oospores. These oospores are of the same type and fall within the range of size recorded for *P. palmivora* and *P. parasitica*. The criteria on which the species under investigation are classified are critically examined and it is found that no significant differences exist between them. The three species are able to grow at 35° C.

The readiness with which these combine to form oospores shows their specific affinity. It is argued that all the three species should be combined into one and the name *P. colocasiæ* is adopted for the emended species as it is the oldest.

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CYTOLOGY OF *COCCINIA INDICA* W. & A. WITH REFERENCE TO THE BEHAVIOUR OF ITS SEX-CHROMOSOMES

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I. Introduction

The family Cucurbitaceæ includes quite a large number of diœcious species. Perhaps on this account it received the attention of the cytologists as early as 1907 when Kirkwood (1907) studied the microsporogenesis of *Micrampelis alba*. Strasburger (1910) first worked on *Bryonia alba* with a view to investigating the mechanism of the diœcious nature of the plant. Sinoto (1928) had observed the presence of a pair of heterochromosomes in *Trichosanthes japonica*; but Bönicke (1911), Lundergardh (1914), Meurman (1925) and Lindsay (1930), working independently with *Bryonia dioica*, failed to find the presence of any heteromorphic pair of chromosomes in this species. Banerji and Das (1937), while studying the meiosis of *Trichosanthes dioica*, did not observe any sex-chromosome pair nor did they obtain any evidence in that direction from a study of somatic mitosis. Nakajima (1937) reinvestigated the meiotic process of *Trichosanthes japonica* and found the presence of a pair of sex-chromosomes as was previously observed by Sinoto (1928). He also recorded the occurrence of a pair of heteromorphic chromosomes in *T. cucumeroides*. Kumar and Deodikar (1940) recorded their observations on the cytology of *Coccinia indica*. They found 26 chromosomes in the somatic nuclei of both male and female plants. Of these, one pair was found to be homomorphic (XX) in the male and heteromorphic in the female (XY). During the meiotic division of the PMCs also, they could observe the presence of a homomorphic pair of chromosomes and noted its behaviour during meiosis. The chromosome number of *C. indica* was previously determined to be $n = 12$ by Sutaria (1936). He, however, had not stated anything about the presence of a pair of sex-chromosomes in this species. It may be pointed out that the chromosome number of the closely allied species *C. hirtella* is also $2n = 24$ (*cf.* McKay, 1930).

A preliminary examination of the somatic nuclei of both the sexes of *C. indica* revealed certain interesting features which did not agree at all with those previously recorded by Kumar and Deodikar (1940). It was found

that the somatic chromosome number of this species as determined by Sutaria (1936) is $2n = 24$ instead of $2n = 26$ as claimed by Kumar and Deodikar. Further, the nature and combinations of the sex-chromosome pair in which they occur in the two sexes were found to be just the reverse of what had been observed by Kumar and Deodikar, *i.e.*, it was found to occur in a heteromorphic (XY) combination in the male and homomorphic (YY) in the female (*cf.* also Bhaduri and Bose, 1947).

From the facts stated above it becomes apparent that a thorough study of the different stages of mitosis and meiosis in both the sexes of *C. indica* is more than necessary in order to arrive at a definite conclusion with regard to the mechanism of sex differentiation in this species. The present paper therefore deals chiefly with the behaviour of the pair of sex-chromosomes at different stages of mitosis in the root-tip cells of both sexes and at the stages of meiosis in the male sex alone of *C. indica*.

II. Material and Methods

The material used in this investigation was collected from plants of *C. indica* growing wild in the Calcutta University College Garden. For the study of somatic mitosis, adventitious roots of both male and female plants, which were obtained from the nodes of scrambling stems, were fixed in La Cour's 2BE fluid which proved to be suitable for the purpose.

For the study of meiosis, flower buds of the male plants were fixed in Nawashin's fluid and Flemming's fluid both of which yielded good results.

After fixation the materials were washed, dehydrated and cleared in the customary way. Sections were cut 8 to 12 μ thick, depending on the stage required for study. Heidenhain's Iron Alum Hæmatoxylin and Newton's Iodine Gentian-Violet stains were chiefly used.

Observations were made with the help of a compensating eyepiece ($\times 10$) and an apochromatic objective (1·2 mm.). Figs. 11 to 14 were drawn at a table magnification of $\times$ 1,600. All other drawings were made with a $\times$ 20 compensating eyepiece and an 1·2 mm. apochromatic objective, giving a magnification of $\times$ 3,700 at the level of the table.

III. Observations

1. *Somatic mitosis*

A study of the somatic chromosomes of both the sexes of *C. indica* revealed that the nuclei of the cells composing the tissue of the male plant are comparatively smaller in size and possess a heteromorphic pair of chromosomes which is absent in the female. In other respects, however, the nuclear

behaviour in somatic mitosis appears to be similar in both the sexes. Hence a common account is presented below, the points of difference being mentioned at the appropriate places.

Resting nucleus—The resting nucleus is comparatively large. Nothing chromatic in nature is visible within the nucleus by the ordinary technique applied except a large nucleolus. The karyolymph however, is not homogeneous. It forms two distinct concentric zones. The zone around the nucleolus is transparent and colourless. The second zone, just outside the first and reaching upto the nuclear membrane, is slightly darker. The nucleolus shows a distinct bud-like process.

Prophase—With the commencement of prophase, small and irregular chromatic bodies are evident in the outer zone of the nucleus (Fig. 1). The size and chromaticity of these bodies gradually increase and they finally assume the structure of typical chromosomes each of which is composed of two intertwining threads. An interesting feature observed at this stage is the presence of a large chromosome in the nuclei of the male plant (Fig. 2, chromosome X). This is absent in the nuclei of the female plant (Fig. 3).

When the nucleus and the chromosomes reach their maximum size, the former gradually shrinks inwards from the two poles, leaving behind two ' polar caps ' of spindle substance (Fig. 4). As the nuclear membrane shrinks more and more around the crowded chromosomes, the ' caps ' become larger and larger and finally assume a definite spindle-shaped figure in which the spindle fibres originate. The double nature of the chromosomes is less evident at this stage due to the increased chromaticity of the chromosomes. Eventually, the nuclear membrane disappears and most of the chromosomes aggregate around the irregular nucleolus which, by this time, has become much reduced in size.

Prometaphase—Prometaphase begins with the disappearance of the nuclear membrane and the organization of the spindle from the ' polar caps '. At this stage the chromosomes condense further and their chromaticity also increases with the result that the double nature of the chromosomes does not become apparent. Soon the nucleolus disappears and the chromosomes lie irregularly scattered over the spindle. At this stage the chromosomes are found to have undergone maximum condensation. Their intertwining nature no longer persists and the two chromatids of each chromosome lie parallel with one another and are seen to be smoother in contour.

Metaphase—The metaphase begins with the orientation of the chromosomes in the equatorial plane of the spindle. Associated with this orienta-

tion the individuality of the two chromatids of a chromosome is no longer evident. A polar view of the metaphase plate shows 24 chromosomes in the preparations of root tips of both male and female plants. But as has been stated before, in the male plant there is a very large chromosome, compared to others all of which are more or less of the same size (Figs. 5 and 6). In the female plant, on the contrary, all the 24 chromosomes are nearly of the same size (Fig. 7).

Anaphase—The movement of the chromosomes is regular and simultaneous (Fig. 8). The two chromatids of each chromosome move to the opposite poles of the spindle where they form the polar clumps (Fig. 9). Due to the compactness of the polar clumps the individuality of the chromosomes at this stage is lost altogether.

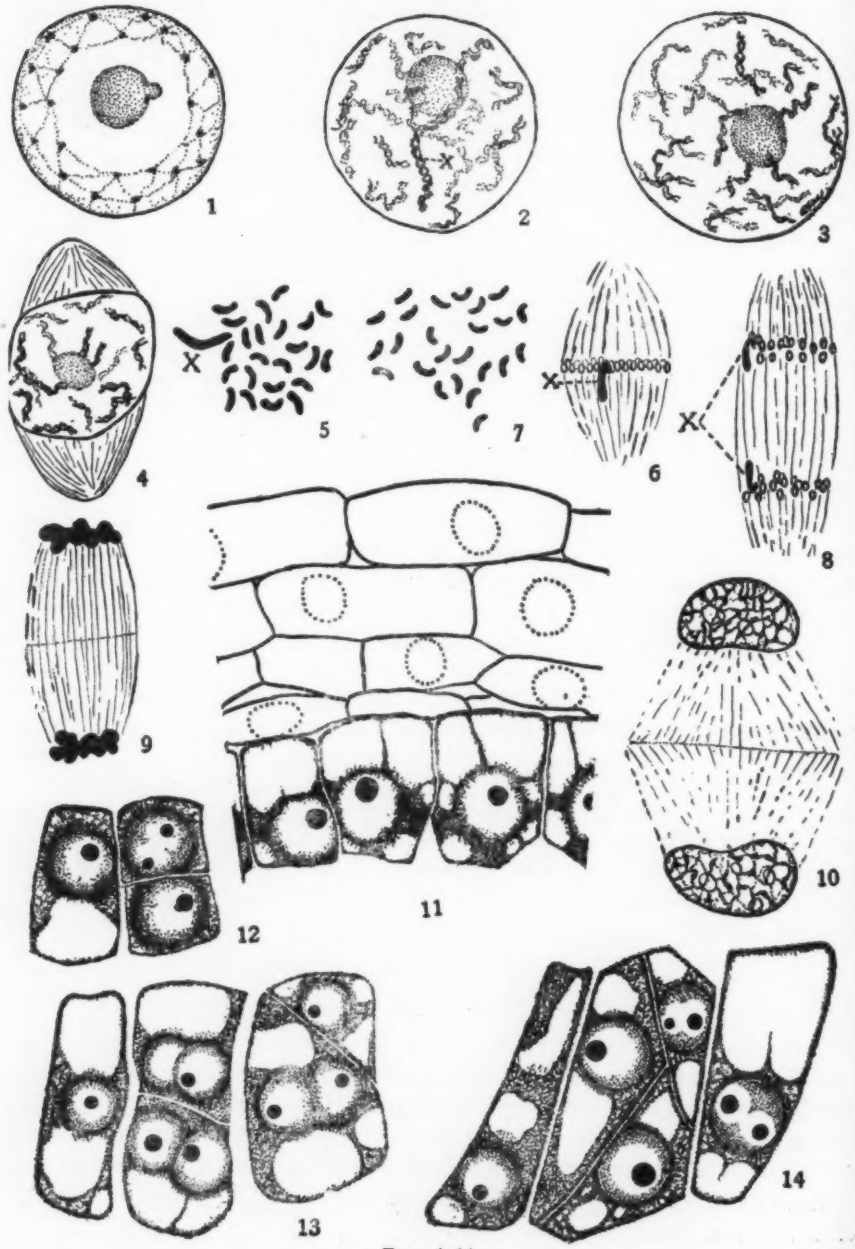
Telophase—Telophase begins with the relaxation of the polar clumps from which the chromosomes emerge out. The chromosomes then appear in the form of slender threads which are thick at certain regions (Fig. 10). They are very soon enclosed by a nuclear membrane. The chromosomal threads gradually become thinner and their chromaticity decreases. Soon the nucleoli make their appearance in each daughter nucleus. Gradually the chromosomes lose their chromaticity and the nucleus appears to be homogeneous except for the large nucleolus. The nucleus at the same time grows bigger in size and assumes the structure described under the resting nucleus.

2. *Tapetum*

The archesporial cells which are hypodermal in origin cut off periclinally a primary parietal layer just below the epidermis and the primary sporogenous cells on the inner side. The primary parietal layer then divides by periclinal walls and forms four layers of cells. Of these, the outermost layer which lies just below the epidermis develops into the endothecium and the innermost one differentiates as the tapetal layer (Fig. 11).

The tapetal cells, to begin with, possess a comparatively conspicuous and large nucleus and deeply staining cytoplasm with large vacuoles (Fig. 11).

During the synizesis of the pollen-mother cells, the nucleus of the tapetal cell divides once only, but this division, as a rule, is not followed by wall formation. Consequently, the two nuclei remain free in the cell. This binucleate condition of the tapetal cells is maintained throughout the development of the pollen grains. In no case has further division or fusion of these nuclei been observed.



FIGS. 1–14

FIGS. 1–14.—Figs. 1–10. Stages in somatic mitosis in the root-tip cells of *C. indica.* Fig. 1. Early prophase. Figs. 2 and 3. Late prophase nuclei of male and female plants. Note the chromosome X in Fig. 2. Fig. 4. Polar caps. Fig. 5. Metaphase plate of male plant. Note the chromosome X. Fig. 6. Metaphase of the same in side view. Fig. 7. Metaphase plate of female plant. Fig. 8. Anaphase of male plant showing the chromosome X. Fig. 9. Polar clump. Fig. 10. Telophase. Fig. 11. Uninucleate tapetal cells. Fig. 12. Two tapetal cells in one of which nuclear division has been followed by cytokinesis. Figs. 13 and 14. Different portions of tapetum showing different characters of individual tapetal cells.

It is interesting to note that in a few tapetal cells nuclear division is followed by cytokinesis leading to the formation of two uninucleate cells (Fig. 12). Further divisions of these cells sometimes take place giving rise to binucleate daughter cells or uninucleate grand-daughter cells as will be seen from Figs. 13 and 14.

Cooper (1933) after a thorough study of the nuclear behaviour in tapetal cells of 7 monocotyledonous and 36 dicotyledonous species has formulated three main groups based on the nuclear phenomenon of tapetal cells. These are as follows:—

Group I—in which the mature tapetal cell is uninucleate,

Group II—in which the nucleus divides once only mitotically and the binucleate condition is maintained throughout the development of the pollen grains,

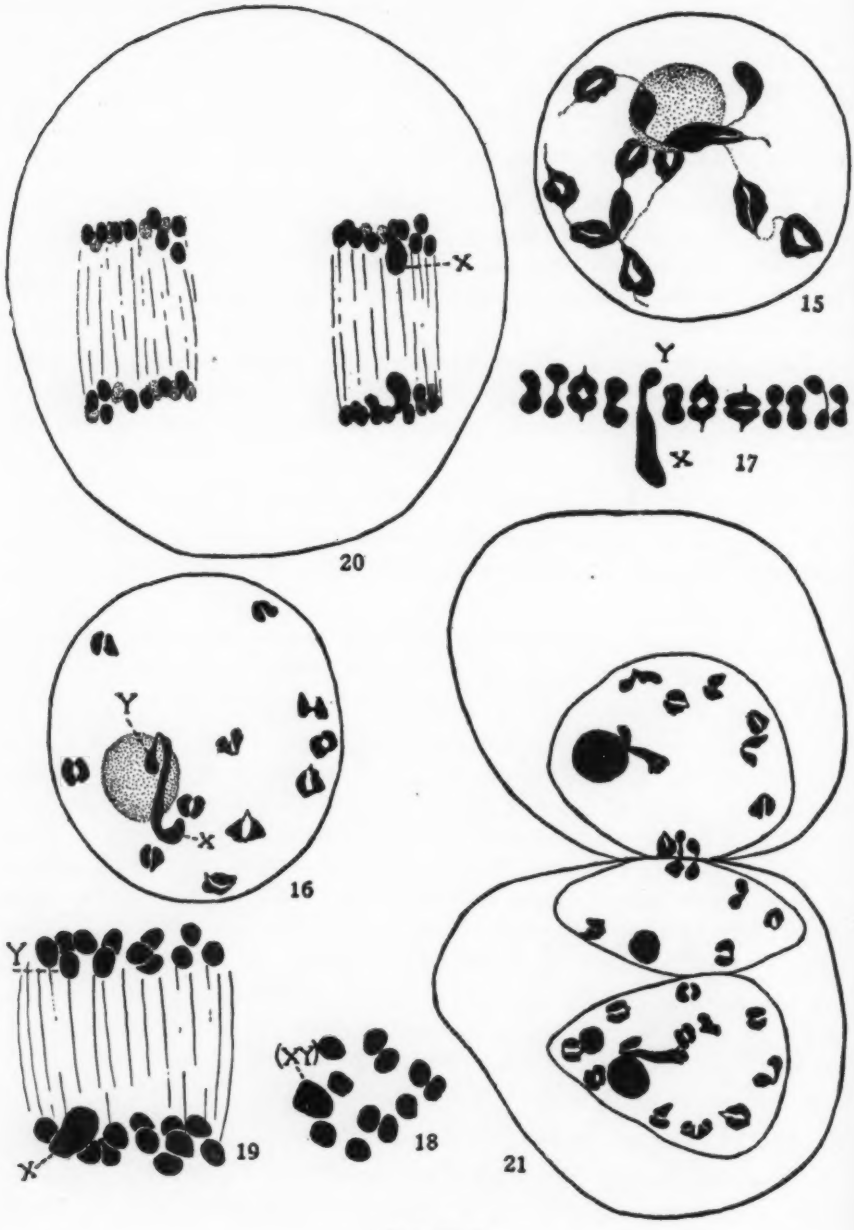
Group III—in which the cells are multinucleate as a result of more than one mitosis. In this group, those plants in which the cells remain uninucleate or binucleate through abnormality in divisions, are also included.

Referring to the above classification it appears that *Coccinia indica*, strictly speaking, does not belong to any of these three groups because the divisions of nuclei of some of its tapetal cells are followed by cytokinesis (Figs. 12 to 14), a condition which has not been recorded by Cooper (1933) and is not found in any of the three groups formulated by him. Nevertheless it shows resemblance to Group II more closely than to the other two, as the tapetal cells or their derivatives attain the binucleate condition by a single mitosis and which condition is retained thereafter.

The two nuclei in the tapetal cell are at first somewhat apart from each other, but soon they come quite close and lie either side by side or one above the other. This approximation of the two nuclei is sometimes so close that they may lie in actual contact with one another (Fig. 13).

3. *The behaviour of chromosomes during meiosis*

The earlier stages of meiosis pass through quite regularly. The presence of the sex-chromosome pair could not be observed until the nuclei



FIGS. 15–21

FIGS. 15-21. Stages of meiosis in PMCs of *C. indica*. Fig. 15. Early diakinesis. Fig. 16. Typical diakinesis showing the heteromorphic pair (XY) attached to the nucleolus. Fig. 17. Side view of metaphase I showing XY pair. Fig. 18. Polar view of metaphase I. Fig. 19. Anaphase I showing disjunction of XY. Fig. 20. Anaphase II. Fig. 21. Binucleate PMC and cytomixis.

reached the diakinetic stage. As such the behaviour of the chromosomes have been described from diakinesis onwards.

Diakinesis—The diplonema threads open out a little and shortly afterwards the bivalents become organised. As the chromosomes shorten and thicken the chromatic elements of the diplonema threads appear to retract from the two ends and condense at the centre leaving behind achromatic threads which later disappear. Fig. 15 shows twelve such condensed masses of chromatic matter which represent the haploid number of chromosomes of the plant. Gradually the chromosomes condense still further and the linin connections between them become obscured. At this stage the twelve bivalents are visible in the nuclear cavity; of these one pair is distinctly heteromorphic a member of which is much larger than any of the members of the homomorphic pairs. The heteromorphic pair in most of the nuclei has been found to be associated with the nucleolus (Fig. 16).

Metaphase I—The bivalents take their position at the equatorial plane of the spindle (Fig. 17). A polar view just before anaphasic separation does no longer exhibit the double nature of the bivalents which assume a more or less rounded form. Among the twelve bivalents, one is distinctly larger than the rest (Fig. 18). The largest one evidently represents the heteromorphic pair observed in diakinesis.

Anaphase I—Anaphasic separation of the univalents begins soon. Some of them assume V-shaped forms, thereby suggesting that these have a median spindle attachment region. The heteromorphic pair becomes clearly marked out on account of the unequal size of its members, as will be seen from Figs. 17 and 19. The movement of the chromosomes is more or less regular and simultaneous. Often one or more univalents are seen to be cast off from the spindle. The univalents on reaching the two opposite poles form the polar clumps in which the identity of the chromosomes is lost. Later the chromosomes emerge out from the polar clumps as long threads and form two interkinetic nuclei.

Division II—The two interkinetic nuclei lose their membranes and their nucleoli disappear. Two bipolar spindles are organised and the two haploid groups of chromosomes which showed evidence of longitudinal splitting in the interkinetic nuclei arrange themselves at the equatorial region of the spindles. At this stage their longitudinal split becomes obscured.

Anaphasic movement soon commences and this is simultaneous in both the spindles. On reaching the poles the chromosomes lose their identity. Very soon a nuclear membrane is organised and the nucleoli make their appearance.

In one of the spindles of the second division the presence of the largest chromosome could easily be made out, but the presence of its homologue in the other could not be determined due to the almost equal size of the chromosomes of the spindle in question. Fig. 20 shows the two spindles during anaphase II. In one of these the presence of the largest chromosome may be noted.

The four nuclei formed as a result of the second division thus differ both qualitatively and quantitatively. Two of the nuclei which receive one longitudinal half of the largest chromosome are alike, so far as this chromosome is concerned, but differ from the other two which are again alike in possessing one longitudinal half of the homologue of the largest chromosome.

Cytokinesis—Cytokinesis takes place by furrowing. The arrangement of the pollen quartets is generally tetrahedral but isobilateral tetrads have also been noted. Quadripartition of the microspores by furrowing appears to be the rule in Cucurbitaceæ (*cf.* Asana and Sutaria, 1932; Castetter, 1926; Heimlich, 1927; Banerji and Das, 1937; Passmore, 1930).

4. *Irregularities during meiosis*

The following abnormalities have been observed during the meiotic division of the pollen mother cells in *Coccinia indica.*

(i) *Cytomixis*—The phenomenon of cytomixis was first described by Gates (1911) while studying the microsporogenesis of *Oenothera gigas.* Katterman (1933) recorded the various stages of cytomixis in PMCs of *Triticum* × *Secale* hybrids. The same phenomenon was observed by Nandi (1937) in a variety of rice during the stages of meiosis and also by Banerji and Das (1937) in *Trichosanthes dioica.* Binucleate pollen mother cells have been noted by Gates and Rees (1921) in *Lactuca*, Karpechenko (1927) in the hybrids of *Raphanus* × *Brassica* and Kihara and Lilienfeld (1934) in certain hybrids between *Triticum* × *Aegilops.* Banerji (1940) working on *Tridax procumbens* observed multinucleate pollen mother cells.

In *Coccinia indica* binucleate pollen mother cells have been observed at almost every stage of development. The origin of these cells might be due to the union of cytoplasm of two pollen-mother cells or to a process of cytomixis or by the absence of wall formation during premeiotic division. In

all probability the last explanation seems to be more plausible but the occurrence of cytomixis is also not uncommon in this species. Fig. 21 illustrates a condition where one of the pollen mother cells contains two nuclei in diakinetic stage and the other only one such nucleus. It will be evident from this figure that an actual transference of chromatin is taking place between them. A similar condition has been figured by Banerji and Das (1937) in *Trichosanthes dioica.*

(ii) *Polyspory*—It is a common feature of *Coccinia indica* that, during the first meiotic division, one or more univalents are cast off from the spindle during anaphase. The cast-off chromosomes then organize a separate small nucleus in the cytoplasm of the pollen-mother cell. Hence in such pollen-mother cells three interkinetic nuclei are formed after the first division. Each of these nuclei divides again. The six resulting nuclei in the pollen-mother cell after cytokinesis give rise to six pollen grains, but the two developed from the cast-off chromosomes are much smaller in size. Such abnormality in pollen grain development has been recorded by a number of workers and Stebbins (1932) is of opinion that the irregularities in meiosis which lead to polyspory are due to heterozygosity.

IV. Discussion

Among those who believed that sex determination is influenced by some internal conditions are Cuénot (1899) for animals and Strasburger (1900) for plants. The theory was subsequently strengthened by the discovery that in many animal and plant species the two sexes differ in their chromosome complements.

Since 1923, several species of diœcious angiosperms have been shown to be characterised by the possession of a heteromorphic sex-chromosome pair. Sinoto (1928) observed the behaviour of the sex-chromosomes in a number of diœcious monocotyledonous and dicotyledonous plants. Kihara (1929), Sinoto (1929) and Lindsay (1930) reviewed the subject of sex-chromosomes in higher plants and gave lists of diœcious plants whose sex-chromosomes had been studied. Nakajima (1937) observed the behaviour of the sex-chromosomes in 16 species of angiosperms. Besides these, a number of diœcious angiosperms have been studied with a view to determine the sexual mechanism involved.

It is well known that sex-chromosomes occur in male and female organisms in different combinations. In plants, these are mostly of the XY type as have been previously observed by Allen (1917, 1919) in Sphærocarpus, Heitz (1928 *a* and *b*) in *Pellia Neesiana* and by Santos (1923, 1924) in *Elodea canadensis.* Recently, a similar type of sex-chromosome pair

has been recorded in a number of angiosperms. Ono (1930) has observed the peculiar form of sex-chromosomes Y X Y or XYn type in *Rumex acetosa*.

Coccinia indica also shows XY type of sex-chromosomes. In connection with somatic mitosis it has been stated that the somatic chromosome number of both male and female plants in 24, of which one pair is heteromorphic in the male and obviously homomorphic in the female, although the homomorphic pair of the female could not be differentiated from the other chromosomes of the complement. But by a study of the behaviour of the unequal pair of chromosomes in the male plant during microsporogenesis and by a comparison of the size of the largest chromosome of the male plant with any one of the chromosomes of the female it can be definitely stated that the male contain 22 autosomes + XY and the female, 22 autosomes + YY.

It has been stated in connection with meiosis that two of the pollen grains of a quartet differ qualitatively from the other two, because two of them contain the X chromosome + 11 autosomes and the other two contain the Y chromosome + 11 autosomes. All the eggs produced by the female plant are qualitatively alike so far as the sex-chromosome is concerned, because each of them contains a Y chromosome + 11 autosomes. Hence the union between a male gamete developed from a pollen grain containing the X chromosome and an egg will produce a staminate plant and union of an egg with a male gamete developed from a pollen grain containing the Y chromosome will produce a pistillate plant.

Recently Kumar and Deodikar (1940) have made some observations on the somatic chromosome complement of both male and female plants and the behaviour of the sex-chromosomes during microsporogenesis of *Coccinia indica*. Their observations however, appear to be altogether different from those made during the course of the present investigation. According to them there are 26 chromosomes in the somatic nuclei of both male and female plants. Of these, one pair is homomorphic (XX) in the male and heteromorphic (XY) in the female. Hence according to them all the pollen grains or the male gametes are qualitatively alike and there are produced two kinds of eggs.

As regards the chromosome number of *Coccinia indica*, the author's observation agrees with that made by Sutaria (1936) and Bhaduri and Bose, (1947) who independently determined it to be $n = 12$. In this connection it may be mentioned that the somatic chromosome number of the closely related species, *C. hirtella*, is also $2n = 24$ (*cf.*, McKay, 1930).

As regards the origin of the sex-chromosomes of *Coccinia indica* during microsporogenesis, Kumar and Deodikar observed an interesting feature.

According to them the sex-chromosomes do not appear in the nuclear cavity until the autosomes undergo considerable longitudinal contraction and reach the late deplotene stage. They state, " From the persistent nucleolus an outgrowth arises which later extends out to form a coiled structure. Still later this detaches itself to form the homomorphic pair of sex-chromosomes each with a secondary terminal constriction ".

The observations and interpretations made by the present writer regarding the origin and behaviour of the sex-chromosomes during the microsporogenesis of *C. indica* seem to differ very markedly from those made by Kumar and Deodikar. In this connection it has been observed that the pair of sex-chromosomes which is heteromorphic and not homomorphic, differentiates along with the other eleven autosomes and that though it has been found to remain attached to the nucleolus, no evidence has been obtained in support of Kumar and Deodikar's observation that it originates from the latter. In recent years (Gates, 1939) it has been reported that the sex-chromosome pair is nucleolar, and as such the significance of its association with the nucleolus (*cf.* also Bhaduri and Bose, 1947) as has been observed during the present investigation, becomes apparent.

V. Summary

A study of the somatic mitosis of *Coccinia indica* has shown that there are 24 chromosomes in the nuclei of both male and female plants, of which one pair is heteromorphic (XY) in the male and homomorphic (YY) in the female.

The somatic metaphase spindle originates from the polar caps which appear as a result of the shrinkage of the prophase nucleus.

During synizesis of the pollen mother cell the tapetal cells become binucleate. In certain cases the nuclear divisions are followed by cytokinesis. The two nuclei of the daughter cell again divide, and the later nuclear divisions may or may not be followed by wall formation.

At diakinesis of the pollen mother cell 12 bivalents have been seen, of which one is heteromorphic and has been found to be attached to the nucleolus.

A polar view of metaphase I shows 12 bivalents. One of these is decidedly bigger than the others and is the sex-chromosome pair (XY), whose identity could be followed during most of the later stages of meiosis.

Various forms of abnormalities have been noted, among which cytomixis and the binucleate condition of the pollen mother cells appear to be the most common.

Laggards have been found to be characteristic of the species. The lagging univalents organize micronuclei which subsequently divide along with the dyad nuclei, resulting in polyspory.

VI. Acknowledgement

In conclusion the writer takes this opportunity of expressing his thanks to Dr. I. Banerji under whose guidance this work was carried out in the Botanical Laboratory of the Calcutta University.

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LEPTOCEPHALI OF THE GULF OF MANAAR*

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In view of the scanty records of Indian Leptocephali (Kaup, 1856; Southwell and Prashad, 1919; Deraniyagala, 1934; Gopinath, 1946 and Nair, 1946 and 1947), a thorough and systematic study of these larvæ together with a knowledge of their distribution is an important and necessary prelude to the study of the biology of the Indian eels of which we know so little while great advances have been made in the study of their European counterpart.

During the course of a three days' visit to Krusadai Island, Gulf of Manaar, early in July, 1947, numerous specimens of Leptocephali were collected each day from the shore seines operated by fishermen from Kutikal Point of Rameswaram Island. On the 8th July 1947, the larvæ occurred in enormous numbers composed of two varieties of *Congrellus anago* and *Uroconger lepturus*. It may be mentioned here that *Stolephorus commersonii* and *Chirocentrus dorab* formed the predominant catch of fish in the shore seines during these days. As may be expected while using such nets, all the specimens were unfortunately taken in a dead or dying condition and consequently no observations on their metamorphosis could be made.

Congrellus anago (Schlegel)

6th July 1947	..	10 specimens.
7th July 1947	..	11 specimens.
8th July 1947	..	72 specimens.

The Leptocephalus of *Congrellus anago* was recorded recently by Gopinath (1946) from the Trivandrum Coast. Apparently the identification of the larva is based on circumstantial evidence afforded by the presence of the elvers of *Congrellus anago* along with the Leptocephalus and, therefore, confirmation of Gopinath's correlation appears to be necessary.

Measurements.—Total length 142 mm.; length of head 4 mm.; length of trunk 110 mm.; length from anus to tip of tail 28 mm.; length from tip of snout to origin of dorsal fin 120 mm.; maximum height 13·5 mm.

The Leptocephalus is transparent, long and flattened with 116 relatively broad myotomes 1·5 mm. wide near the middle region of the body. A

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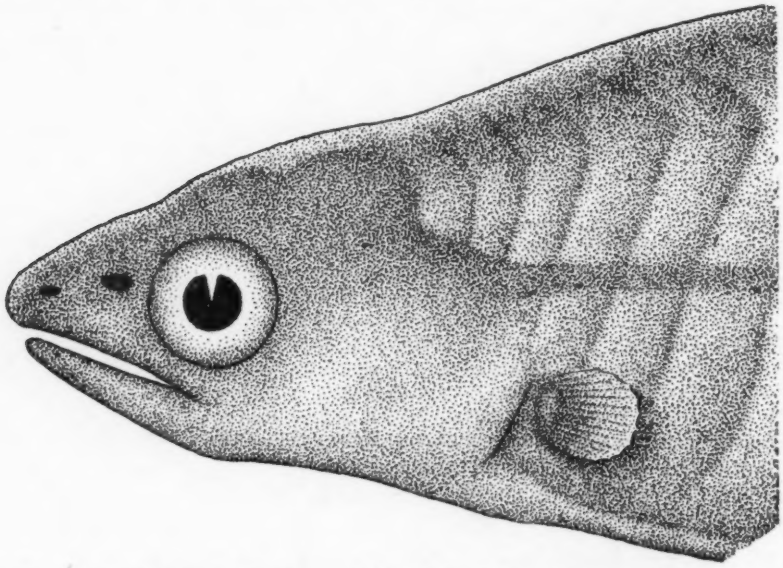
narrow transparent region to which the myotomes do not extend is present on the ventral side above the alimentary canal. The small, triangular head has a bluntly pointed snout (Fig. 1). The upper jaw is slightly longer than



TEXT-FIG. 1. Head region of the Leptocephalus of *Congrellus anago*. × *ca* 18.

the lower and in all the specimens the larval teeth have fallen off. No indication of the formation of the adult set of teeth is seen in any of the larvæ. The cleft of the mouth is straight and extends posteriorly to the level of the centre of the eye. The alimentary canal is very long and straight and forms a prominent gradually broadening region between its commencement and the 24th myotome. While in a few of the specimens the anus is placed below the 82nd myotome, in the majority, however, it has shifted anteriorly, and this fact taken in conjunction with the absence of the larval set of teeth suggests that the larvæ have reached their limit of full growth and have just begun to metamorphose into the elvers. It is possible that in the fully grown larva, before the onset of metamorphosis, the anal opening may be situated under a still posterior myotome. The dorsal fin is very short with closely-set developing fin-rays while the anal is slightly longer with the rays well developed and prominent. The caudal fin is confluent with the vertical fins whose rays indistinguishably merge with those of the former.

The head is devoid of pigmentation. The presence of black stellate chromatophores at the bases of all but a few of the anteriormost rays of the anal

fin and of the caudal fin appears to be a distinguishing feature of the larva of *Congrellus anago*, and this pigmentation is clearly visible even to the naked eye. Similar chromatophores are present at the bases of the better developed posterior fin-rays of the dorsal fin. Elongated irregularly arranged black chromatophores are present along the dorsal side of the alimentary canal. In some specimens, a row of black chromatophores is found along the ventral half of the myocommas of the posterior half of the body. These groups of pigment cells become prominent towards the caudal end.

It is known that the Leptocephalus of *Congrellus anago* occurs in good numbers from the beginning of November till the end of February along the Trivandrum Coast (Gopinath, 1946). This larva, which appears to be the common Leptocephalus of the Gulf of Manaar, has not so far been recorded along the Madras Coast in the regular plankton collections made at the University Zoological Research Laboratory, Madras, during the last ten years. But we know that the recorded habitat of the adult is from the Coromandel Coast of India to Malay Archipelago (*Congromuræna anago*, Day, 1889). We are thus confronted with certain interesting problems about the distribution of the larva and the adult of *Congrellus anago*.

Uroconger lepturus (Richardson)

6th July 1947	..	3 specimens.
7th July 1947	..	11 specimens.
8th July 1947	..	21 specimens.

An account of the Leptocephalus of *Uroconger lepturus* occurring in the Madras plankton and the correlation between myotome and vertebral counts of the larva and the adult respectively has already been given (Nair, 1946).

All the larvæ in the present collection are edentulous with great reduction in the length of the alimentary canal and the height of the larva which varied from 8–9 mm. These changes indicate that the larvæ are in a more advanced stage of metamorphosis than those collected from the Madras plankton. This is corroborated by the appearance of new chromatophores in the head region consequent on the commencement of metamorphosis. Five to six black stellate chromatophores are present in a horizontal line near the heart region with one or two stray pigment cells above them. Two or three similar chromatophores occur at the middle region of the upper jaw.

The Leptocephalus of *Uroconger lepturus* can, therefore, now be taken as common on the East Coast of India. *Leptocephalus acuticaudatus* was collected by Kaup (1856) from Malabar and though the descriptive account of the larva is meagre and unsatisfactory, Kaup's figure of it bears a strong

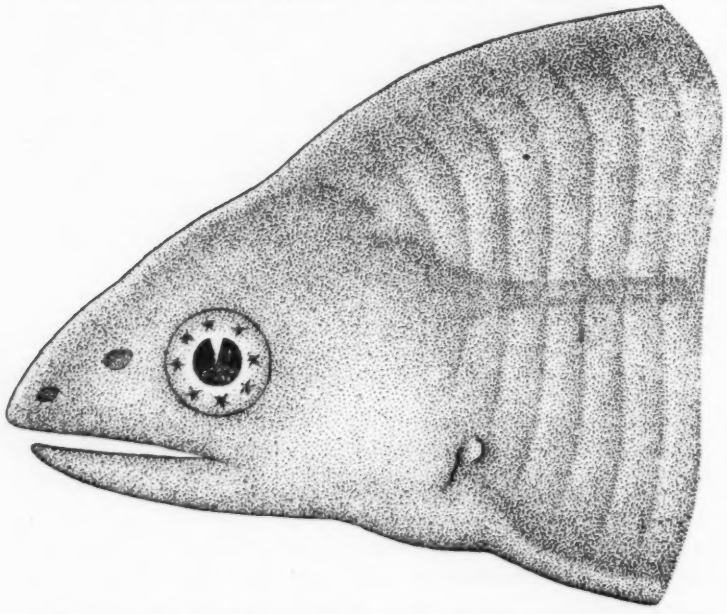
resemblance to the present larva in certain features. Therefore, it is possible that *Leptocephalus acuticaudatus* may belong to the genus *Uroconger*.

Muræna sp.

6th July 1947 .. 1 specimen.

Measurements.—Total length 66 mm.; length of head 4 mm.; length of trunk 43 mm.; length from anus to tip of tail 19 mm.; length from tip of snout to origin of dorsal fin 38 mm.; maximum height 9 mm.

The relatively small, flattened and leaf-like larva has 210 myotomes. The head is conical with a pointed snout (Fig. 2). The gape of the mouth



TEXT-FIG. 2. Head region of the Leptocephalus of *Muræna* sp. × *ca* 18.

is straight and extends to a level with the middle of the eye. The larval set of teeth has fallen off and no indication of the formation of the adult set is present. The course of the alimentary canal is straight with the anal opening below the 126th myotome. The pectoral fin is present as a very small and rudimentary structure.

The eye is tinged yellow with eight black stellate chromatophores round the pupil. The body is devoid of chromatophores except for the presence of a few minute black ones at the bases of the posterior region of the anal and the caudal fins.

The larva very closely resembles that of *Muræna macrura* described by the author (1947) from the Madras plankton; the coloration of the eye particularly is very striking, and it is probable that the larva belongs to a species of *Muræna* not unrelated to *Muræna macrura*. Deraniyagala (1934) has recorded two larvæ belonging to Murænidæ from Ceylon waters. Kaup's (1856) figure of *Leptocephalus dussumieri* which was collected from Malabar and imperfectly described by him reminds one of a Murænid larva. Many Murænid larvæ which look alike not only in appearance but also in other characters should be expected from the Indian Seas where many species are known to occur.

Muræsox cinereus (Forskål)

Three specimens of the Leptocephalus of *Muræsox cinereus* collected on the 3rd May 1947, by my colleague, Mr. P. R. Sadasivan Tampi, from a shore seine worked from Kutikal Point were handed over to me with the information that *Dussumieria hasseltii* formed the bulk of the catch in the net.

An account of the larva of *Muræsox cinereus* together with the changes undergone by it during metamorphosis into the adult has previously been given by the author (1947).

The Leptocephalus of *Muræsox cinereus*, like that of *Uroconger lepturus*, can also now be considered as common on the East Coast of India.

My thanks are due to Dr. H. Srinivasa Rao, Chief Research Officer, for going through the manuscript and making helpful suggestions in improving the format of the paper.

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PRELIMINARY TRIALS IN THE PROPAGATION OF *BRACON (MICROBRACON) GREENI* ASHMEAD ON UNNATURAL HOSTS

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Introduction

Mass propagation of parasites and predators is of great practical importance in biological control work, and their breeding for large-scale releases to a great extent depends on a continuous and adequate supply of host material. In most cases it is not possible to have a sufficiently large supply of the natural host at all times of the year, so that it becomes imperative to find out alternate hosts on which the parasites could be bred in the laboratory. There are numerous instances wherein alternate hosts have been employed successfully for an artificial rearing of the parasites although they may not have been recorded from these hosts under natural conditions, as for example, the mass breeding of the egg parasite, *Trichogramma evanescens minutum* on the eggs of various lepidopterous pests of stored products and the breeding of the braconid, *Macrocentrus ancylivorus*, a larval parasite of the Oriental fruit moth, on the potato tuber worm.

The control of the two major predator enemies of lac, *Eublemma amabilis* and *Holcocera pulverea* on a mass scale by utilising their natural enemies has been engaging the attention of this institute since 1930. Results of the biological control experiments carried out during 1941–1944 have already been published (Negi, Gupta, Misra, Venkatraman and De, 1945) and papers on the mass breeding of *Bracon greeni* on natural and alternate (unnatural) hosts were read at the annual session of the Indian Science Congress by Gupta (1938) and Venkatraman (1945) respectively. The present paper gives an account of the studies on the olfactory responses of the parasite in relation to its mass production, together with the result of preliminary attempts in the propagation of *B. greeni* in the laboratory on some unnatural hosts (Annual Reports, 1930–45).

Host Selection by *Bracon greeni*

General behaviour of parasite.—*B. greeni* Ashm., is a primary, ectophagus, larval parasite of the lac predator, *Eublemma amabilis* Moore,

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occurring in the major lac growing areas in India (Misra, Negi and Gupta, 1930). The biology and habits of the parasite have been studied in great detail by Gupta. The parasite is specific in the selection of its host and so far has not been found to parasitise any other host in nature.

The female parasite, as soon as it comes in the vicinity of a host, is attracted towards it, and this initial attraction of the parasite to the host is mainly due to the odour of the host larva. Having reached the region in which the host is located, the female parasite tries to find out the exact spot by vibrating its antennæ. The olfactory sense appears to be located in the antennal segments; adults devoid of antennæ or adults having their antennæ coated with shellac varnish, seem to evince little interest for oviposition. When once the parasite is within the reach of the host, the texture of the dome-shaped covering of the host larva, the size of the host larva and its movement while the parasite tries to sting it, probably give the final stimulus to oviposit. The parasite deposits its eggs upon or near the host after paralysing the larva with a toxic fluid while stinging. The braconid parasitises the middle and later stages of *E. amabilis* larva.

Odour of the host and its web, the chief attracting factor.—Preliminary studies on oviposition response of the female parasite to stimuli such as size, texture and odour of the host showed that odour emanating from the host larva and its associated spun envelope was the dominant factor in host selection. Hence the responses of the parasite to this factor alone were investigated by means of a modified McIndoo's olfactometer devised by Thorpe and Jones (1937). The results detailed in Table I to IV confirm the conclusions drawn from preliminary experiments.

Females used in these experiments were bred in the Laboratory and had not previously oviposited, but had copulated. They were mostly about 2 to 3 days old and were fed with cane sugar solution. All precautions to avoid any possible errors were taken. The experiments were carried out in a dark room, where the temperature and humidity were fairly constant. The position of the objects used for testing odour, was alternated from one arm to the other at each trial and the olfactometer was thoroughly washed after every three experiments.

Results of Experiments

1. The degree of attraction exerted by smell of *Eublemma* larva was tested first. Eight medium-sized larvæ of the natural host were removed from their galleries and kept in one arm of the apparatus, the other arm being left blank. Fifty gravid females which had not oviposited previously, were used in each of these experiments. Results are presented in Table I.

TABLE I

Response of *Bracon* (reared on *Eublemma*) to *Eublemma* larva in olfactometer

Sr. No. of Expts.	No. of females entered in the arm of the olfactometer			Remarks
	Containing *Eublemma*	Blank	Total	
1	10	6	16	Out of 186 choices the arm containing *Eublemma* was favoured by 134 females (72·1%) and the blank arm by 52 females (27·9%). The standard deviation calculated from the formula $Sd = \sqrt{\frac{S(x-\bar{x})^2}{N \times N-1}} = \pm 2{\cdot}28$ Results considered significant since the value 72·1% exceeds 27·9% by more than twice the standard deviation
2	17	5	22	
3	10	8	18	
4	21	12	33	
5	15	3	18	
6	18	4	22	
7	12	3	15	
8	24	11	35	
9	7	0	7	
Total	134 = 72·1%	52 = 27·9%	186	

2. Next, the intensity of attraction exerted by the odour of the web spun by *Eublemma* larva on *Bracon* reared from *Eublemma* was tested. Fresh webs of larva lined with pellets of its excreta were placed in one arm and the other was left blank. Fifty individuals were used in each of these experiments.

TABLE II

Response of *Bracon* (reared on *Eublemma*) to the web spun by *Eublemma* larva, in olfactometer

Sr. No. of Expts.	No. of females entered the arm of olfactometer			Results
	Containing *Eublemma* web	Blank	Total	
1	11	7	18	Out of 291 choices, *Eublemma* web was preferred by 203 females (69·8%) as against the blank arm by 88 (30·2%). Standard deviation $Sd = \pm 2{\cdot}7$ Results considered significant.
2	9	7	16	
3	20	3	23	
4	18	4	22	
5	29	11	40	
6	15	8	23	
7	7	7	14	
8	12	6	18	
9	26	11	37	
10	8	9	17	
11	31	5	36	
12	17	10	27	
Total	203 = 69·8%	88 = 30·2%	291	

3. Here the reaction of *Bracon* to a choice of odour of *Eublemma* larva and its web was studied. Eight larvæ of *Eublemma* were placed in one arm and fresh larval webs of *Eublemma* in the other. Forty-five females were used.

TABLE III

Response of *Bracon* (reared from *Eublemma*) to *Eublemma* larva and larval web, in olfactometer

Sr. No. of Expts.	No. of females entered the arm of olfactometer containing			Results
	Eublemma larva	*Eublemma* web	Total	
1	10	11	21	Out of 262 choices *Eublemma* larva was preferred by 121 females (46·2%) as against *Eublemma* web preferred by 141 females (53·8%). Standard deviation $Sd = \pm 1{\cdot}97$
2	12	17	29	
3	16	10	26	
4	11	18	29	
5	14	16	30	
6	21	13	34	Results significant.
7	15	16	31	
8	8	11	19	
9	5	21	26	
10	9	8	17	
Total	121 = 46·2%	141 = 53·8%	262	

4. The reaction of the third generation of *Bracon* reared on *Platyedra gossypiella* to a choice of two hosts, *viz.*, *Platyedra* and *Eublemma* was investigated. Eight larvæ of *Eublemma* were placed in one arm and eight *Platyedra* larvæ in the other, care being taken to use larvæ of about the same size. Twenty-five gravid females reared continuously for three generations on *Platyedra* were used in each of these experiments.

TABLE IV

Responses of *Bracon* (rared on *Platyedra* for three generations) to larvæ of *Platyedra* and *Eublemma*, in olfactometer

Sr. No. of Expts.	No. of females entered in the arm of olfactometer containing			Results
	Eublemma	*Platyedra*	Total	
1	8	7	15	Out of 119 choices, *Eublemma* was preferred by 74 females (62·1%) *Platyedra* by 45 (37·9%) Standard deviation $Sd = \pm 1{\cdot}45$
2	10	4	14	
3	11	10	21	
4	4	4	8	
5	13	4	17	Results significant.
6	9	7	16	
7	6	2	8	
8	13	7	20	
Total	74 = 62·1%	45 = 37·9%	119	

Breeding of *Bracon greeni*

1. *Host material.*—The natural host, *Eublemma*, is not easily obtained in large numbers from the field, throughout the year. During the months, March to May, it is scarce in the field, because most of the eggs laid by the third generation of moths from October to February on *Baisakhi* (October to June) and *Aghani* (June to January) lac crops, do not develop due to cold and those laid by the moths of the fourth generation that survive winter are greatly affected by heat in summer months (Misra, Negi, Gupta, 1930). Attempts to rear the host on a large scale in the laboratory have not been successful so far. On account of these difficulties the breeding of parasites on unnatural hosts was undertaken.

Under Laboratory conditions, *Bracon greeni* has been successfully bred on many unnatural hosts, the criterion of success being judged by the development of parasite grubs to maturity. A list of hosts tried so far is given below, the hosts being arranged according to the classification of Simmonds (1944).

List of Unnatural Hosts Tried

Category I. Attack without much reluctance: subsequent development normal.

Platyedra gossypiella (pink boll worm of cotton), *Scirpophaga nivella* (top borer of *sugarcane*) and *Leucinodes orbonalis* (*Brinjal fruit borer*).

Category II. Attack with reluctance: subsequent development subnormal.

Trachylepidia fructicassiella (*Cassia fistula* pod borer), *Enarmonica perfricta* (*Pongamia glabra* seed borer), *Corcyra cephalonica* (rice moth), *Chilo zonellus* (maize borer), *Etiella zinckenella* (pea borer), *Plutella maculipennis* (diamond back moth), *Ephestia cautella* (*Bassia latifolia* seed borer), *Cataglyphus bicolor* (red ant larva).

Category III. No attack : no progeny

Earias spp. (*Hibiscus esculentus* fruit borer), *Earias* spp. (spotted boll worm of cotton), *Gnorimoschema operculella* (potato tuber worm), *Hypsiphyla robusta* (*toon* shoot borer), *Bombyx mori* (mulberry silkworm), *Emmalocera depressella* (root borer of sugarcane).

A summary of results of breeding *Bracon greeni* on unnatural hosts during 1943–1946 is presented under Table V.

2. *Breeding technique.*—The method devised by Negi and Gupta for the mass breeding of parasites on the natural host *Eublemma* cited and used by Glover and Chatterji (1936) is being followed to breed the parasites on unnatural hosts also, but the mode of presentation of host larva has been modified.

A. Methods of offering host larvæ for parasitisation.—Several methods were tried to offer the natural host *Eublemma* for parasitisation and from the view-point of maximum parasitisation the following three methods have been found most suitable in the order of merit.

(1) Small cut pieces of lac sticks containing healthy *Eublemma* larva *in situ* (Fig. 7). This method, though gives the highest parasitisation, is not always feasible, because, while cutting the *Eublemma* infested portions of lac twigs a considerable part of healthy lac is also likely to be removed from the plants.

(2) *Eublemma* larva are collected by picking from lac-bearing trees or harvested lac and each larva placed singly in a bit of lac called ' dome ' (Fig. 1). The larva spins a fine web of silk and excreta over it. Such ' domes ' are termed ' lac domes ' (Fig. 6).

(3) Each *Eublemma* larva placed singly in a lac bit, is covered with a thin tissue paper (bleached sulphate paper about 0·02 mm. in thickness). These ' domes ' are called ' tissue domes ' (Figs. 2, 3 and 4).

The cut bits of lac or ' lac domes ' or ' tissue domes ' containing *Eublemma* larvæ are fixed to a stick having a coat of plasticene over it and these artificially made lac sticks or pseudo-sticks are presented for parasitisation.

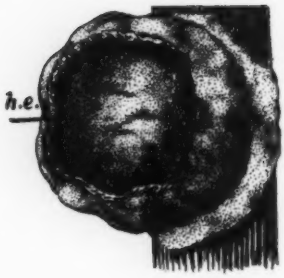
In the case of unnatural hosts, for obvious reasons, method (1) is not possible, hence trials were given to methods (2) and (3) and various other devices, but only method (3) described above was found promising. But it lacked sufficient attraction to the parasite and in the case of agile larvæ, such as of *Trachylepidia fructicassiella*, most of the larvæ escaped from the ' tissue domes ' by biting out holes in the tissue paper cover. The larvæ that came out escaped parasitisation and constructed webs inside the oviposition cage, with the result that adult parasites often got entangled in the web. Therefore, methods had to be evolved to make the host larvæ inactive and acceptable to the parasite.

B. Artificial paralysation of host.—The two most successful methods were (1) amputation of mouth parts without injury to head capsule, and (2) coddling, by immersing the larvæ in hot water at a particular temperature for a certain time. The former method is laborious and requires skill which may be had with practice. In the case of the latter method, the temperature of water and the duration of treatment have to be determined for each species. There was some mortality of host larvæ, which were paralysed by the above methods, but pupation was prevented in most cases.

TABLE V. *Summary of breeding* B. greeni *on unnatural hosts during* 1943–46

Name of host	Mode of exposing the host larva	Host larvæ exposed to action of parasite			Host parasite contact in days	Adult parasites bred					Remarks on host suitability
		No. exposed	No. parasitised	Percentage parasitism		No. of males	No. of females	Total adults	Percentage of females	Adults per host larva	
1	2	3	4	5	6	7	8	9	10	11	12
1 *Platyedra gossypiella* (pink boll worm of cotton)	In 'tissue dome'; tissue covering studded with *Eublemma* excreta; not paralysed artificially.	37,451	12,967	34·6	1–5	4,923	5,992	10,915	54·9	0·8	A suitable host to breed *B. greeni*; the larva remains quiet inside 'domes'. It can be stored in infected cotton seeds for some time.
2 *Trachylepidia fructicassiella* (*Cassia fistula* pod borer)	In 'tissue domes'; covering pasted with *Eublemma* excreta ; larva quietened by coddling or amputation of mouth parts.	6,526	893	13·6	1–5	213	362	575	62·9	0·6	A suitable host if available in large numbers. Requires artificial paralysation to stay in 'tissue domes'. Oviposition technique requires improvement.
3 *Enarmonica perfricta* (early seed borer of *Pongamia glabra*)	In 'tissue domes', tissue covering containing *Eublemma* excreta; coddled before presentation.	2,400	938	39·1	1–4	74	150	224	67·0	0·2	The parasite grubs do not seem to relish the juice of caterpillars, which have a repugnant odour. Attempts are being made to breed this host on edible pods to change the odour.
4 *Scirpophaga nivella* (top borer of sugarcane)	In 'tissue domes', tissue paper covering with *Eublemma* excreta; not paralysed artificially.	97	29	29·9	1–4	19	31	50	62·0	1·7	Larva remains quiet inside 'domes' and full grown larva can subsist as many as 6 parasite grubs. Larva from second instar onwards suitable for breeding the parasite. It would be an useful substitute host, if sufficient and continuous supply could be ensured.

5 *Corcyra cephalonica* (the rice moth)	In 'tissue dome'; tissue covering studded with *Eublemma* excreta; coddled (47° C. for 6 minutes) before presentation.	474	88	16·8	1–4	20	43	63	68·2	0·8	Larva very active and has many small hairs which seem to be disliked by broconid; requires artificial paralysation to stay in 'domes'. Easy to breed in the laboratory in abundance.
6 *Leucinodes orbonalis* (brinjal fruit borer)	In 'tissue dome'; tissue covering pasted with *Eublemma* excreta.	29	6	20·9	1–4	1	2	3	66·6	0·5	It will serve as a useful laboratory host, but the incidence of the pest is very low in and around Ranchi.
7 *Chilo zonellus* (maize borer)	In 'tissue dome'; tissue paper covering pasted with '*Eublemma* excreta coddled (46°C. for 5 min.) before exposure.	77	6	7·7	2–4	1	1	2	50·0	0·3	Larva thick skinned and active requires artificial paralysation. Further investigation is considered necessary.
8 *Cataglyphus bicolor* (ant larva)	In 'tissue dome'; no artificial paralysation necessary.	1,006	17	1·7	2–5	1	10	11	90·9	0·6	Heavy mortality of host larvæ inside oviposition cage, hence not suitable for mass breeding.



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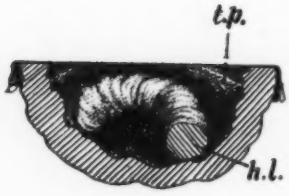

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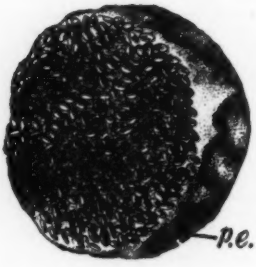

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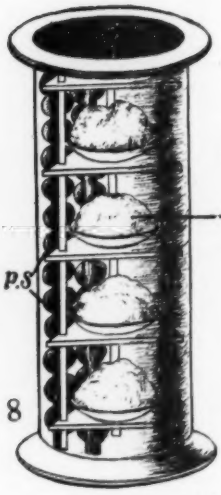

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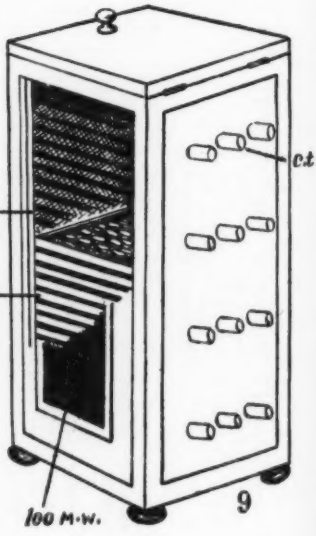

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FIGS. 1—9

(All figures diagrammatic)

FIGS. 1-9.—Fig. 1. 'Dome' of lac, enlarged. Fig. 2. 'Tissue dome,' enlarged. Fig. 3. 'Tissue dome,' partially opened to show host larva, enlarged. Fig. 4. Transverse section of 'tissue dome,' enlarged. Fig. 5. 'Tissue dome' with *Eublemma* excreta pasted over tissue covering, enlarged. Fig. 6. 'Lac dome,' naturally spun by *Eublemma* larva, enlarged. Fig. 7. *Eublemma* spun dome, *in situ*, on lac stick, enlarged. Fig. 8. Oviposition cage. Fig. 9. Emergence cage.

c. s. cotton swabs soaked in sugar solution.
c. t. collecting tubes.
h. e. hollow excavation in 'dome'
h. l. host larva.
p. e. pellets of *Eublemma* excreta.
p. s. 'psuedosticks' bearing 'domes'.
s. t. storage trays.
t. p. tissue paper covering.
100 m.w. 100-mesh wire-gauze on outer wall.
15 m.w. 15-mesh wire-gauze on inner wall.

(*a*) *Rendering host larva suitable for acceptance.*—The next attempt in the method of presentation was to make the tissue paper covering of 'domes' simulate the natural web of *Eublemma* in regard to odour and texture. It has been stated earlier that the odour of the natural host, *Eublemma* and its web and excreta is a dominant factor in the initial attraction of the female parasite to the host, wherefore, fresh pellets of excreta of *Eublemma* larva were pasted one layer thick to the tissue covering of 'domes' (Fig. 5). Tissue 'domes' containing unnatural hosts with and without *Eublemma* excreta were introduced in a series of identical oviposition cages, and out of 1,910 *P. gossypiella* larvæ offered in tissue domes without *Eublemma excreta*, 18·8% were parasitised and out of 2,560 *P. gossypiella* larvæ offered in tissue domes covered with *Eublemma* excreta 39·3% were parasitised. The results obtained clearly show that better oviposition results are obtained by pasting *Eublemma* excreta over this tissue covering of the 'domes'. This conclusion is further confirmed by the experiments conducted on 14,931 *E. amabilis* larvæ (natural host) offered in tissue domes without *Eublemma excreta* over them and 37,451 *P. gossypiella* larvæ (unnatural host) offered in 'tissue domes' pasted with *Eublemma* excreta; the percentage of parisitisation in the former was 29·6 and in the latter 34·6 *i.e.*, 5% more.

It is a little tedious to collect large quantities of *Eublemma* excreta; consequently, a mixture of pellets of *Eublemma* excreta and finely powdered stick lac instead of pure *Eublemma* excreta was experimented on the 'tissue domes' and more satisfactory results were obtained than with finely powdered stick lac alone.

(*b*) *Oviposition cage.*—Cylindrical glass jars, $12'' \times 4\frac{1}{2}''$ with 80-mesh wire-gauze covers (Fig. 8) serve as very handy and suitable breeding cages (Gupta, 1938). Into each cage about 30 to 40 adult females and about half the number of males are introduced. 3% to 5% sugar solution is pro-

vided in the cage as food for adults. About fifty to sixty 'domes' containing host larvæ are introduced in each cage. The cages are kept in a well illuminated place and the 'tissue domes' were removed from them to storing cages, 1 to 5 days after introduction, depending on the season.

(*c*) *Storing of parasitised host larvæ.*—The 'domes' from the oviposition cage are stored in the emergence cage in trays (Fig. 9). The emergence cage consists of a double-walled box, 10″ × 12″ × 16½″. The two outer side walls are fitted with 100-mesh wire-gauze screened by black cloth which can be removed or put on at will, the front wall has round holes to fix specimen collecting tubes, and the back side has a door to push the trays in. The inner three walls of the cage are of 15-mesh wire-gauze which permits the emerging braconids to pass through easily and prevents the unparasitised host, if any, from emerging out. The space between the inner and the outer walls is 2″ on three sides and 1″ on the back; this allows sufficient ventilation to the developing parasites. Each cage has 15 trays and each tray can hold 100 to 150 'domes'. The trays and outer walls of the cage on the inner sides are painted dull black, while the whole cage is painted white on the outside. The emerging parasites collect themselves in the glass tubes fitted in the front side of the cage, where they mate and are periodically collected.

Discussion

Flanders (1944) observed that 'there is little doubt that odour may be critical factor in the mass production of certain species of parasitic hymenoptera, and by the manipulation of odours the reproduction of such insects may be either stimulated or repressed.' The experiments conducted to study the responses of *Bracon greeni* to the odour of the host, clearly indicate, that the smell of *Eublemma* larva and its associated spun web is an important factor in the acceptability of the host by the parasite. Of the odours emanating from the larva and the web containing excreta, the smell of the latter appears to offer more attraction to the ovipositing female.

It was also found that at least for 3 generations, rearing on unnatural hosts did not seem to result in an actual preference for the new host over the natural one. These observations are in conformity with the results of Thorpe and Jones (1937). Much has been written on this aspect of host selection by parasitic species and recently Simmonds (1944) has discussed this problem in detail and has concluded that "there is no apparent segregation of a strain particularly adapted to the unnatural host, and it would appear that the possibilities of such breeding interfering with the success of the introduction of a parasite species is remote."

The method of breeding insect parasites on unnatural hosts constitutes an important problem in biological control work. The successful breeding

of a parasite in the laboratory depends mainly upon a close study of its habits and behaviour, especially its responses to oviposition stimuli, which in most cases are found to be highly selective. In the case of *Bracon greeni*, although odour appears to play an important part in the acceptability of the host, there seems to be several other factors which operate not singly, but in combination. The main characteristics of hosts acceptable to *B. greeni* are that, (1) they must be sluggish, smooth and thin skinned, with very few bristly hairs, (2) they must be at least many times the size of the parasite, and (3) they must be surrounded by a thin rigid covering of a particular texture, with a little space between the body of the host and the covering.

ACKNOWLEDGMENTS

Our thanks are due to Mr. J. C. M. Gardner, Forest Entomologist, Dehra Dun, for offering helpful criticisms; to Mr. K. C. Chatterji of the department for his assistance in the breeding work and to Mr. E. Heber, Artist, for preparing the illustrations. Our appreciations are due to Dr. P. K. Bose, the Director of the Institute, for his interest in the work.

SUMMARY

1. This paper presents an account of the olfactory responses of the parasite, *Bracon* (*Microbracon*) *greeni* in relation to its mass production, together with the results of preliminary attempts in the breeding of the parasite on some unnatural hosts. *B. greeni* is a primary ectophagous larval parasite of the lac predator, *Eublemma amabilis* Moore.

2. With a view to finding out a suitable laboratory host for the mass breeding of the parasite, the nature of oviposition response of the female parasite produced by such stimuli as size, texture and odour of the host larva was studied. Experiments conducted with an improved McIndoo's olfactometer, indicate that the smell of the natural host (*Eublemma*) and its associated spun web plays an important part in the initial attraction of the parasite. Of the odours emanating from larva and the spun web, the smell of the latter appears to offer more attraction to the ovipositing female. It was also observed that rearing on unnatural hosts did not seem to result in an actual preference for the new host over the natural one.

3. The technique in the breeding of *B. greeni* on unnatural hosts is described in some detail. Host larvæ are exposed to the action of the parasite in ' tissue domes ' (lac bits covered with tissue paper), the tissue paper covering being studded with pellets of *Eublemma* larval excreta These tissue covering pasted with excreta simulated the natural web of *Eublemma* and offered sufficient attraction to the ovipositing female. A mixture of

pellets of excreta and finely powdered stick lac also gave satisfactory results. The larvæ of active unnatural hosts invariably escaped out of 'tissue domes' in which they were presented for parasitisation, by biting out the tissue paper covering. Of the methods tried for making the larvæ inactive, the most successful were (1) amputation of mouth parts without injury to head capsule, and (2) coddling, by immersing the larva in hot water at a particular temperature for a certain time.

4. Although *B. greeni* is a specific parasite of *E. amabilis*, it has been bred successfully in the laboratory on a number of unnatural hosts, most of them being pests of economic importance. A list of unnatural hosts tried is given. Results of breeding on unnatural hosts are presented.

Of the hosts tried in the laboratory, *P. gossypiella* (pink boll worm of cotton) appears to be the most suitable host. *S. nivella* (sugarcane top borer) will serve as an additional substitute host if a regular supply of this host material could be ensured. *T. fructicassiella* (*Cassia* pod borer) and *C. cephalonica* (rice moth) also seem promising.

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A NOTE ON SOME ABNORMAL FLOWERS OF *HIBISCUS ESCULENTUS* L.

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(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

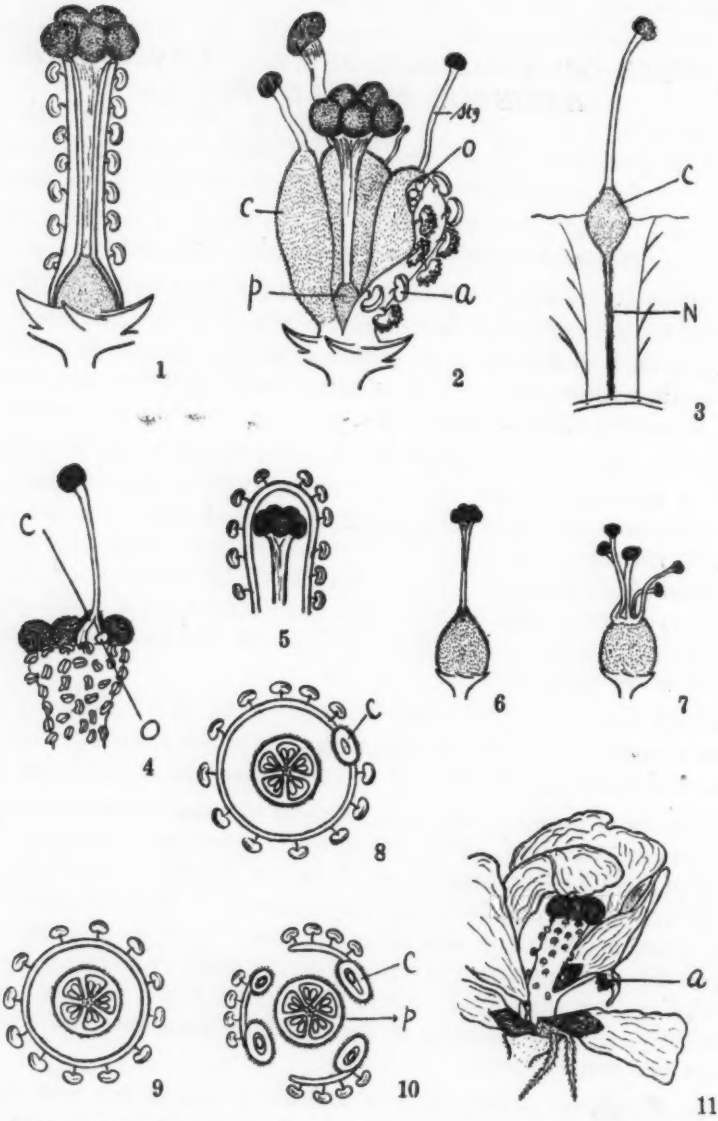
Some interesting examples of abnormalities in the floral structures in plants of several natural orders have been recorded from time to time. Many such cases have been recorded in some species of *Gossypium* and *Hibiscus*; but, as far as the author is aware, only very few instances of such abnormalities in the flowers of *Hibiscus esculentus* have so far been reported upon.

The occurrence of intracarpellary fruits has been recorded by Delavaud (1858) in *Hibiscus tiliaceus*, and by Harris (1913) in *Hibiscus esculentus*. Such an instance has also been reported by Bergman (1932) in some hybrid forms of *Hibiscus*. The presence of extra-carpellary outgrowths has also been recorded in *Hibiscus esculentus*. Saksena (1932) found a few outgrowths from the syncarpous fruits of this species, and Venkataramani (1945) observed a second rudimentary ovary with a separate style and stigma of its own in a flower of the same species.

Wilcox and Holt (1913) and Bergman (1932) have recorded another type of abnormality in some hybrid *Hibiscus* in which a second flower was produced through an extension of the central axis of the primary flower.

Instances of petalody have been recorded in some species of *Gossypium* and *Hibiscus* (Sankaran, 1931; Ramanatha Ayyar and Sankaran, 1934; and Singh, 1935). The production of stalked stigma-like structures from the staminal tube of the flower has been recorded in *Hibiscus esculentus* by Venkataramani (1945). In this abnormal flower from the free end of the staminal tube were produced two thread-like structures resembling the style, each ending in a stigma-like body. The same flower showed another abnormality, *viz.*, the doubling of the epicalyx—there were two whorls of epicalyx one over the other.

The occurrence of carpellody in *Hibiscus esculentus* has been recorded by Agharkar (1927). In all the cases of carpellody observed by him one to three of the topmost stamens of the staminal tube were modified into carpellary structures. According to him, the basal part of the filament became



Text-Figs. 1–11.—Diagrammatic sketches showing the abnormalities in some flowers of *Hibiscus esculentus* L.

Fig. 1. Showing a section of the staminal tube of a normal flower and the pistil. Fig. 2. Staminal tube modified into three portions with carpel-like structures. *a*. anther;

o. ovules; *stg.* style and stigma; *p.* normal pistil in the middle of the three staminal portions; *c.* carpel-like structure. Fig. 3. A portion of the staminal tube with a stamen modified into a carpel-like structure (viewed from inside the staminal tube). *C.* broadened carpellary portion on the staminal tube; *N.* narrow, slender portion of the carpel with hairs extending to the base on the inner surface of the staminal tube. Fig. 4. A part of the staminal tube with a number of stamens on the outer surface and the modified carpellary structure. *C.* carpel; *O.* exposed ovule. Fig. 5. Staminal tube completely covering the stigmatic lobes. Fig. 6. A normal pistil with a single stylar column and five separate stigmatic lobes at the top. Fig. 7. An abnormal pistil with five separate styles, each ending with a separate stigma. Fig. 8. Diagrammatic sketch of the staminal tube in section showing a single stamen modified into a carpel (C). The normal ovary is in the centre. Fig. 9. Diagrammatic sketch of the staminal tube and ovary of a normal flower. Fig. 10. Diagrammatic sketch of the staminal tube and ovary of an abnormal flower showing the staminal tube modified into three portions. C. carpel-like structure; *p.* normal ovary. Fig. 11. Flower showing petalody. Note the position of the petaloid structure. *a.* anther lobe showing a few pollen grains.

broader and bore one or two ovules on the margins, thus resembling an open carpel; the middle part of the filament developed into the style while the anther was replaced by the bushy stigma.

The writer came across five types of abnormal flowers in *Hibiscus esculentus* during the years 1946–47 in the experimental plots of the University Botany Laboratory, Madras. As these types of abnormal flowers do not appear to have been recorded previously they are briefly described below in this communication.

1. *The complete covering of the stigmatic lobes by the staminal tube.*—This was observed in a flower (variety, " Podugu ") which had been bagged for self-pollination. The flower was found shed, and on examination it was found that the stigmatic lobes were completely enclosed within the staminal tube (Plate III, Fig. 9; Text-Fig. 5) and were, therefore, prevented from being pollinated. The shedding of the flower was evidently due to the failure of pollination.

2. *Separation of the styles.*—This type of abnormality was met with in a flower of another variety, " American Long Green ". There were five separate styles, each with a stigma of its own and all the five styles arising from the top of the ovary, unlike in the normal flower where there is a single stylar column which divides only at the top into five or more separate stigmatic lobes. In this abnormal flower four of the stigmatic lobes were observed to come out tearing through the staminal tube (Plate III, Figs. 7 and 8; Text-Figs. 6 and 7).

3. *Petalody.*—Two instances of petalody were observed by the author in the flowers of *Hibiscus esculentus*. In both the cases one of the stamens was modified into a petaloid structure (Plate III, Figs. 1 and 3). In one of

these two cases an anther lobe with apparently normal pollen grains was found on the margin of this modified structure (Plate III, Fig. 2; Text-Fig. 11). In the second flower, however, no such anther lobe was found.

4. *An instance of Carpellody.*—An interesting case of carpellody was observed in a flower of the variety P-15 originally obtained from the Punjab. In this abnormal flower the staminal tube was split lengthwise into three portions (Plate III, Figs. 5 and 6; Text-Fig. 2). In two of these three portions one of the stamens was modified into a carpel-like structure, while in the third two stamens were similarly modified into carpellary structures (Text-Fig. 10). In each of the two former portions the modified carpel was broad and elongated and was attached to the staminal tube portion on one side. The staminal tube portion was normal with the usual large number of stamens. The new carpel-like structure had a long style ending in a large and round stigma. The upper portion of the carpel was slightly open exposing some of the ovules. The outer surface of the carpellary portion was covered with hairs as in the normal carpel of the flower.

In the third portion of the divided staminal tube two stamens were modified into carpel-like structures and between these two was seen the attached staminal tube portion (Text-Fig. 10). One of the carpels on the staminal tube portion was large and similar to the carpel of the other two portions of the staminal tube with the upper portion slightly open exposing some of the ovules. The other carpel was smaller and had a very short style with a small stigma at the top. This carpel was not open but was completely closed.

This abnormal flower differed from the normal one in that the staminal tube was modified to contain within the normal style and five stimagtic lobes at the top (Plate III, Fig. 6; Text-Fig. 2).

5. *A second instance of Carpellody.*—A second instance of carpellody was observed in a flower of another variety, "Pal". In this case one of the stamens of the staminal tube was modified into a carpel-like structure. The staminal tube was complete and otherwise quite normal (Plate III, Fig. 4; Text-Fig. 4). When the staminal tube was split open the small modified carpellary structure was seen slightly broadened at the top of the staminal tube with the lower portion of the carpel very narrow and continued downward on the inner face of the staminal tube (Text-Fig. 3). It had a long style terminated by a capitate stigma. The surface of the carpel and its narrow lower portion were covered with hairs as in the normal carpel of the flower. The broadened upper portion of the carpellary structure was open

towards the outside above the level of the staminal tube exposing a single ovule (Plate III, Fig. 4; Text-Fig. 4).

This modification resembles to a certain extent the one recorded by Agharkar (1927). But in the case of Agharkar's specimens one to three stamens were modified into carpels, whereas in the present case only a single stamen was modified into a carpellary structure.

It may be interesting to note that all these abnormal flowers were observed on old plants at the end of the growing season. Agharkar (1927) has also mentioned that the flowers showing carpellody observed by him were almost always borne on old plants.

Acknowledgments

The author's thanks are due to Dr. T. S. Sadasivan, M.Sc., Ph.D., Director, University Botany Laboratory, Madras, and to Prof. M. O. P. Iyengar, M.A., Ph.D., F.L.S., for their helpful criticisms and suggestions in the preaaration of this paper.

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Explanation to Plate III

Fig. 1. Flower showing petalody.

Fig. 2. An enlarged view of Fig. 1. Note the position of the petaloid structure and the anther lobe with some pollen grains (A).

Fig. 3. Another flower showing petalody. Note the position of the petaloid structure on the staminal column.

Fig. 4. A portion of the staminal tube with one of the stamens modified into a carpel-like structure. Note the open carpel with a single exposed ovule (o).

Fig. 5. An abnormal flower showing the staminal tube modified into three portions.

Fig. 6. Another view of Fig. 5 showing the modified staminal portions and the normal pistil in the middle of the three staminal portions.

Fig. 7. The abnormal flower showing four stigmatic lobes coming out tearing through the staminal tube.

Fig. 8. A normal pistil, and the abnormal pistil with five separate styles each with a stigma of its own.

Fig. 9. Flower showing the staminal tube completely covering the stigmatic lobes.

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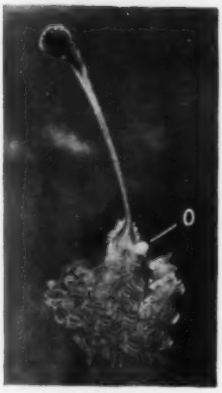

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NUTRITION IN THE ADVANCED EMBRYOS OF THE SCORPION: *PALAMNAEUS SCABER* THORELL.

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Received August 30, 1947

(Communicated by Dr. K. L. Moudgill, F.A.Sc.)

During my study of the development of the scorpion *Palamnæus scaber* Thorell, I came across a peculiar feeding mechanism in the later embryos. Unlike the heavily yolked eggs which are characteristic of most scorpions, the Scorpioninæ to which *Palamnæus* belongs have yolk-free eggs and from the earliest period of development the embryos in these are nourished from maternal tissues. This aspect has been studied by Laurie (1891), Poljansky (1903), Pavlowsky (1924) and Pflugfelder (1930), but these works have been confined more to the earlier stages.

In the later stages in the embryonic development of *Palamnæus* when the bulk of the embryo has considerably increased and nutritional demands are great, a highly specialised type of feeding apparatus is perfected, of which I give below a brief account.

The developing embryo of *Palamnæus* lies in a diverticulum of the ovarian tube (Fig. 1). When the egg is fertilised and begins to segment, this diverticulum is only 1·7 mm. long, but soon it undergoes remarkable changes. It rapidly elongates, enlarges and its apex, beyond the position of the embryo, grows rapidly into an elongated process, which has been named by Laurie (1891) the "appendix" (Fig. 1, *apx.*). The following measurements would serve to indicate the rapid enlargement of the diverticulum and its appendix.

Stage of embryo	Length of Diverticulum	Length of Appendix	Total length
	mm.	mm.	mm.
Fertilised egg beginning to segment	1·7	..	1·7
4 blastomere stage ..	3	4·5	7·5
Segmentation completed ..	4	9	13

An advanced embryo in relation to its diverticulum and appendix is shown in Fig. 2. The embryo is 12 to 13 mm. long, has all the parts fully formed and, except for some details, is a little scorpion lying compactly

packed, in the highly stretched diverticulum. No vital connection of the embryo with the maternal follicular wall is noticeable.

The " appendix " lies free in the hæmolymph. Its distal end which may be called the *end piece* (*ep.* Figs. 3 and 4*A*)—" endknopchen " of Pflugfelder (1930)—consists of a mass of clear cells which take up vital stains readily (Fig. 5*B*). It forms an absorbing organ. Its cells absorb nutriment

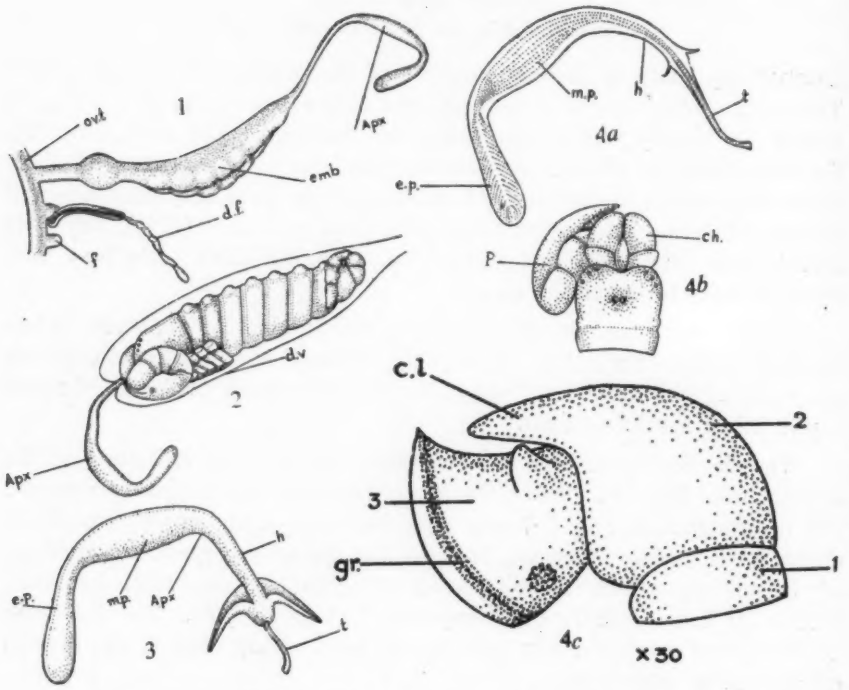


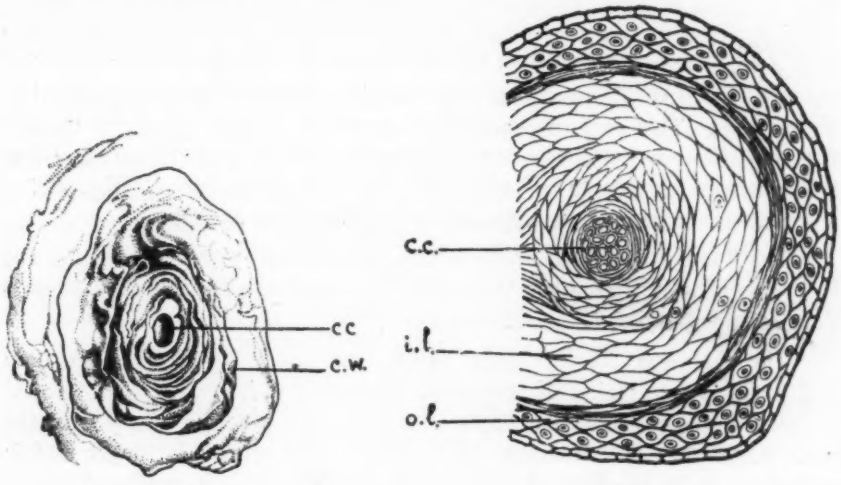
FIG. 1. Developing embryo of *P. scaber* in its diverticulam × 12. *apx.* appendix; *d.f.* degenerating follicle; *emb.* embryo; *f.* developing ovarian follicle; *ovt.* ovarian tubule.

FIG. 2. An advanced embryo of *P. scaber* in its diverticulum (dv.). The left wall of the diverticulum is removed so as to expose the embryo *in situ* holding the proximal end of the appendix (*apx.*) in its mouth × 7.

FIG. 3. An appendix removed from the diverticulum of an advanced stage. The proximal end of the appendix (*apx.*) forms the teat (t.) × 15.

FIG. 4 *a*. A schematic diagram of the appendix. *ep.* end piece; *h.* handle; *mp.* the swollen middle region; *t.* the "teat". Fig. 4 *b*. Dorsal view of the anterior end of an advanced embryo. × 9. *ch.* chelicera; *P.* pedipalp. Fig. 4 *c*. A chelicera of an advanced embryo seen from the inner side. × 30. 1, 2, 3. the segments of the chelicera; *cl.* the claw of the second segment; *gr.* the chitinous groove on the inner side of the third cheliceral segment.

materials from the hæmolymph. The cells of the middle region however are arranged to form a clearly marked-out longitudinal core which extend through the middle of the next region also—the "appendixkorper" of Pflugfelder. This region which may be called the *middle piece* (m.p. Figs. 3 and 4 *A*) has the core thicker than the other parts. The cells making up this central core in the appendix are seen to have undergone a special development. In the earlier stages they form a solid rod of cells but in further development their cell walls become considerably thickened and their protoplasmic contents degenerate so as to ultimately form a system of conducting canals. In transverse sections these present an appearance strongly resembling that of plant tissues with their conducting vessels. (Figs. 5*B* c.c.).



5a *5b*

Fig. 5 *a*. Transverse section of the chitinised proximal part of the appendix forming the "teat" × 350. *cc*. central canal ; (in some two or even three canals are noticed) ; *cw*. chitinised wall. Fig. 5 *b*. T. S. of the distal end of the appendix—the end piece. × 200. *i.l.* inner cellular layer ; *o. l.* outer cellular layer ; *cc*. central camals forming the core.

Thus extending from the "*end piece*" through the core of the "*middle piece*" is a system of tiny canals or lacunæ. This lacunæ-bearing core is found to be specially thick in the middle piece which has a swollen appearance and evidently serves as a reservoir for the absorbed fluids.

The proximal part of the appendix as seen externally is narrow and has been termed "steil" (Pflugfelder, 1930) or *handle* (Fig. 3 *h*). The structure of this is essentially the same as the foregoing, differing only in that the central canals are few so that the core here is very narrow. Corresponding

to this the external diameter of this region also is very small. Evidently, the numerous lacunæ of the " middle piece " have converged into these few tubes of this region. Towards the proximal extremity the central core of this region shows a gradual chitinisation of the walls of the tubes.

Beyond this part, the central core of the " appendix " which has already been noticed to begin to get chitinised, continues inside the diverticulum, as a curved chitinised tube, into the hollow of which the several canals of the middle region of the appendix lead (Fig. 5 *A*). This chitinised tube (Fig. 3, *t*) is inserted at the mouth of the fœtus and it is through this tube that the nutriment, absorbed by the appendix, is passed into the mouth of the embryo and so this part may be termed a " *teat* " (Fig. 3, *t*). Examination of living embryos showed that the pharynx with its radial muscles well developed and the gut too, exhibited regular pulsating movements indicating that definite suction is applied by the embryo to the tip of the " teat " of the appendix and that nutriment is definitely sucked in—not merely absorbed by osmosis. This part of the mechanism which I have called the " teat " is not mentioned by Pflugfelder; either he has missed it or it does not occur in *Hormurus australasiæ* on which he made his observations. Fig. 4 *A* is a schematic diagram of the appendix as in *Palamnæus scaber*.

The " teat " described above is held in position by the cheliceræ of the embryo which are developed very early and show special structural adaptations for this purpose (Figs. 4 *b* and 4 *c*).

The cheliceræ formed long before the other appendages are even indicated. When their full embryonic development is reached they project in front of the prosoma and have a curious form differing widely from the adult structure. The first segment is short. The second is longer and is twisted so as to be directed downwards. Anteriorly it is produced into a finger-shaped claw. This claw is, in the adult, placed on the inner side of the appendage but here it is anterior in position. The third segment, which in the adult is outer and forms the movable claw is placed here just behind the anterior claw and has a very characteristic form. Though it is movably articulated it is not claw-like and is not opposed to the anterior claw to form a chela as in the adult. Here, it is a broad, shield-like structure, flat on its inner aspect and convex on the outer. The flat inner aspect is in contact with the corresponding structure on the opposite appendage. Thus the third segment of the two cheliceræ are directed to the median line and meet by their inner, flat surfaces. Between these the chitinous tube or ' teat ' which forms the proximal part of the appendix is held firmly in position. There are chitinous grooves on these opposing surfaces in which the teat lies (Fig. 4 *c*).

During gestation the liver of the scorpion is seen to undergo certain changes. It is comparatively large and occupies the greater part of the body space during the earlier stages of gestation. Estimates of their net weight showed that the liver in these early stages has on an average 15% of the weight of the body. As the embryos develop the liver loses weight rapidly and dwindles in size appreciably. By the time the embryos are fully developed and ready to be extruded, the liver is highly emaciated and represents only on an average 7% of the weight of the body. In such estimates there are considerable chances of error as a recent feed would alter the size and weight of the liver. But in the above estimate care has been taken to avoid such errors as far as possible by taking specimens under more or less similar conditions. This would indicate that the reserve food materials stored in the liver are taken up by the blood and from it the appendices of the ovarian diverticula rapidly absorb them and pass them on to the developing embryos.

A special feature with regard to this feeding mechanism is that it is entirely of maternal origin, the fœtus contributing no part to it. By the time that the zygote begins to segment, this nutritional structure also begins to develop. The only modification in the embryo in connection with this feeding mechanism is an adaptation of its cheliceræ, as mentioned above, for holding the terminal duct or "teat" in position. Laurie (1891) who noticed the appendix of the ovarian diverticulum, correctly recognised it as a feeding mechanism but believed that its "solid core" is being gradually chewed and digested by the fœtus. In the early embryos where this mechanism is not perfected, in place of the chitinous teat-like tube there is a solid rod of chitin-forming cells. This structure as seen in the earlier stages might have suggested this explanation to him.

In typical 'placental' nutrition of fœtuses, capillaries from specialised maternal tissues get into intimate relation with capillaries from the fœtus and thus nutriment from the maternal blood passes into the fœtal blood. In some viviparous fishes there are "appendicula" which absorb nutriment from the uterine fluid but this is not passed into the mouth. In some Elasmobranchs, however, Ranzi (1934) has shown that the uterine secretion is absorbed into the gut through the mouth or spiracles. But even this is only sucking in of the fluids around and a special teat-mechanism is absent. In the peculiar male of the angler fish (*Ceratias holboelli*) which lives as a 'parasite' on the female, a vascular process from the latter fits into the mouth of the former (Regan, 1930). This connection, however, becomes highly vascular and the real passage of nutriment is from the blood of the female to the blood of the male.

Among the viviparous Arthropods there are no instances to be compared with this feeding arrangement. In *Hemimerus* (Heymons, 1912) the growing embryo is nourished by a secretion but this is merely absorbed by the embryo. In *Hesperoctenes* (Hagan, 1931) also, a secretion of the ovarian wall nourishes the embryo but this is absorbed by the " pleuropodial membrane " formed by the growth and fusion of the pleuropodia. In *Glossina* (Roubaud, 1909) there is a milk-secreting gland which opens into the uterus in which the fœtus lies. But nowhere do we have a nutrient fluid-secreting mechanism with a definite teat inserted into the mouth of the fœtus by which the secreted nutrient matter can be directly passed into the alimentary canal of the latter. Finally a remote parallel may be suggested with the condition in a marsupial. But here it is the newly born young that are fed at the maternal teat which is external whereas in the scorpion it is the intrafollicular fœtus that is fed at an internal teat.

Fate of the Feeding Mechanism after the Birth of the Young

The embryos thus richly nourished rapidly attain maturity and are ready to be born. Each now gets detached from the teat and push backwards out of the diverticulum into the ovarian tube. The wall of the ovarian tube is at this time highly elastic and gets distended with the young ones. Their backward propulsion is continued through the ovarian tubules and ultimately one by one they come out.

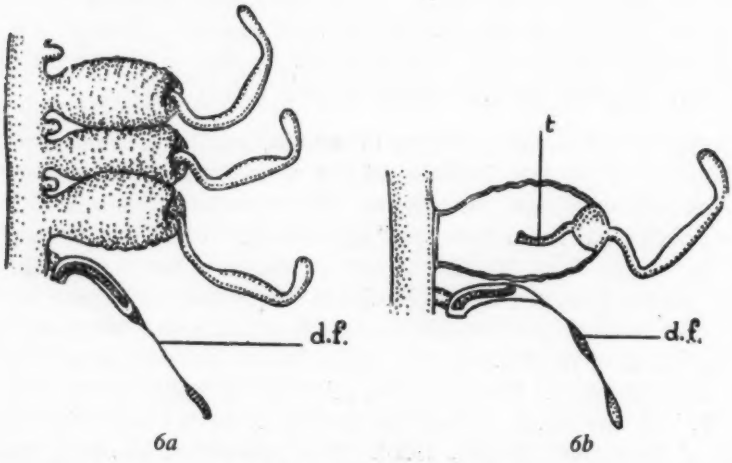


Fig. 6 *a*. Part of an ovarian tube from which the young scorpions have been recently extruded. × 7. *d. f.*, degenerated diverticulum. Fig. 6 *b*. Same as 6 *a* but the wall of the diverticulum opened to show the "teat" (*t*) inside × 7.

After the young have passed out of the diverticulum the latter undergoes remarkable changes (Figs. 6 *a* and 6 *b*). The feeding mechanism is left in the same condition as when it was functioning for the embryo. The teat, now released from the embryo, lies free in the diverticulum which has shrunk considerably and is filled with a fluid. The whole structure soon gets cut off from the wall of the ovarian tube and rapidly undergoes degeneration. A few stages in the breakdown of the diverticulum and appendix are shown in Figs. 6 *a* and 6 *b*. The last part to break down naturally is the chitinous teat, which is seen to persist even after the rest of the diverticulum has disappeared (Fig. 6 *a*).

The newly born young has the peculiar nature of the fœtal chelicera still retained: in this stage it does not and cannot feed but remains mounted on the mother's back waiting for the first moult. At this event, which takes place in a few days, the shield-like third joint is cast off and in its place an opposable claw as in the adult is formed which makes the chelicera a typical chelate structure. After this only, the young one shifts for itself and feeds.

Summary

A highly specialised embryonal nutritional apparatus seen in the embryos of *Palamnæus scaber* Thorell. is described. The eggs are yolkless and their entire development takes place in diverticula of the maternal ovarian tubes. During development each embryo enlarges enormously in bulk and so naturally has to be richly nourished. For this purpose the distal end of the diverticulum is drawn out into an " appendix " which forms an absorbing organ and lies free in the hæmolymph from which nutrient materials are taken up. The middle portion of the appendix appears to serve as a reservoir while its proximal end is a hollow chitinised tube——' the teat "—inserted into the mouth of the embryo. The absorbed nutrient fluids pass into the gut of the embryo through this " teat ". This mechanism thus suggests the arrangement of a " feeding-bottle ".

Some leading types of relationship between viviparous animals and their embryos are recounted to show that there is nothing parallel to this mechanism in any of the well-known examples. At birth the embryos alone come out, the diverticula with the feeding mechanisms being retained in the maternal hæmocoele, where they rapidly undergo degeneration as new diverticula develop.

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ERRATA

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Page	Line	*For*	*Read*
60	12	P. colocasiæi	P. colocasiæ
65	36	*antiquorium*	*antiquorum*
66	10	Dreschter	Dreschler
67	2	*antiquorium*	*antiquorum*
67	3	(1930)	(1934)
67	32	Lenonian	Leonian
67	34	*Omuivora*	*Omnivora*
72	31	*Trans. Brit. Myc.*	*Trans. Brit. Myc. Soc.*
72	34	778. 315	778–815
72	37	— *Ibid.*, 1945, **32**, 509–54.	Chilton *et al.*, *Ibid.*, 1945, **32**, 549–54.
73	4	*Ros. Ser.*	*Res. Sta.*
73	6	384–439.	384–89.

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ISOPOD PARASITES OF FREE-LIVING COPEPODS OF MADRAS

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Received February 21, 1948
(Communicated by Dr. H. Srinivasa Rao, F.A.SC.)

Introduction

Though the Bopyrids of the Indo-Pacific region have been studied by many workers and the Indian Bopyrids treated exhaustively by Chopra (1923), the Epicarids parasitic on free-living Copepods of the Indian coast have not received any attention. Nierstrasz and Brandis (1923) have given an excellent account of the Indo-Pacific Epicaridea collected by Siboga expedition, but the Micronisciдæ parasitic on Copepods have been given only a brief reference. Tattersall's account (1905) of the Micronisciдæ collected from Nordisches Plankton has however proved very helpful to the authors in preparing this brief note on three of the Indian forms, occurring on the Madras coast.

In the course of the studies of Copepods of Madras Plankton, 13 Isopods were found as ecto-parasites on free-living Copepods belonging to the genera *Acartia*, *Acrocalanus* and *Eucalanus*, between 11–9–1947 and 6–10–1947. The data collected as regards the number of parasites, their respective hosts, size and colour are given in the table below.

Type	No. of Specimen	Colour	Length	Breadth	Host	Remarks
			mm.	mm.		
A	2	Greenish-White with phosphorescent spots	1·64	0·9	Acartia	
B	1	Reddish	1·32	0·48	Eucalanus	
			1·00	0·28	Acartia	
			1·20	0·32	Acrocalanus	
C	7	Greenish-White	1·24	0·36		This is found parasitic on two hosts
			1·32	0·48		
			1·36	0·48		
			1·40	0·56		
			1–24	0·32		
			1–48	0·60		

The hosts were free-living Copepods belonging to the genera *Acartia*, *Acrocalanus* and *Eucalanus*. The parasites do not seem to affect the host

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at all, as far as its swimming powers, body size or sexual maturity are concerned. Nor were there any pathological developments. The percentage of occurrence of the infected Copepods is very negligible. Though a total number of 6,000 specimens of *Acartia* was examined from 20 samples, the number of infected Copepods was only 7. In the same way, among the 10,000 *Eucalanus* and 8,000 *Acrocalanus* examined, only one of the former and two of the latter were found infected.

The 13 parasites collected fall into three definite types and appear to be restricted to three genera of hosts, *Acartia*, *Acrocalanus* and *Eucalanus*, one of the three types being found on two of these hosts (*Acartia* and *Acrocalanus*). As there were no means of determining whether these were larvæ or adults, male or female, the authors felt that they may be provisionally described as different species of the genus *Microniscus* with the specific names indicating the genera of the hosts, as *Tattersall* has done, until we know more about their life-histories.

All the three parasites were found attached to the cephalothorax of their hosts in the same oblique position, the posterior part of the parasite being on the lateral side of the host with the anterior side extending over the dorsal side. This position is probably due to the small size of the hosts—average length 2·97 mm., breadth 0·72 mm., in proportion to that of the parasites—average length 1·45 mm. and breadth 0·56 mm. It is also probable that the oblique position is the most suited for the parasite to be attached without hampering the movements of the limbs of the hosts. Prehension appears to be with the help of the antennæ, the thoracic legs, the flat form of the body and pointed uropods. The parasites are easily dislodged from the body of their hosts and when detached they crawl on their thoracic legs in the watch-glass. Frequently they fall on their dorsal side and remain inactive. Even when the parasites were crawling about, they appeared to be indifferent in securing a fresh host though several Copepods of their host genera as well as others were placed in their vicinity. After dislodging the parasite, the host was examined carefully for any injury inflicted by the mouth parts of the parasite. As there were no wounds in the few forms examined, it is posssible that the parasites had just fastened themselves to their hosts. By the prehensile nature of the limbs and absence of food-capturing organs, the possibility of their being just epizoic can be ruled out. The parasites appear to have no power of swimming, the pleopods which appear to be respiratory never being brought into play. The entire abdomen not being attached to the host, the posterior free part was lifted up and lowered down. Such ventilatory movements were slow, lasting from 8 to 10 seconds and

repeated 5 to 7 times a minute. Observation of the tiny parasites was facilitated by staining them in neutral red. One of them moulted during observation, the ecdysis starting from the cephalic region and extending backwards. But it was found impossible to rear them for a period longer than three days.

Type A

Microniscus acartii

Host: *Acartia erythræa* Giesbrecht.

Locality: Madras Coast.

Size of the Host: 2–7 mm. long and 0·72 mm. broad (average).

The two specimens found, measured 1·64 mm. from the anterior tip of the cephalic plate to the posterior tip of the setæ of the uropod and 0·9 mm. across the body between the outer edges of the tergal plates. The body is twisted about the commencement of the abdominal region so that when the abdomen is viewed dorsally the part anterior to it is seen from a lateral aspect.

The segmentation of the body is very clear, especially on the ventral side. As in all isopods, there are seven free thoracic segments in front of which is a large and prominent shield of crescentic shape. This carapace covers the cephalothorax of six segments. The anterior edge of this plate projects freely forwards for a short length. This free, flexible margin is probably of use in getting a closer and firmer contact on the surface of the host's body than an abrupt sharp edge would secure. The dorsal side of the carapace is marked by a pair of conspicuous eyes consisting of 4 elements. The dorsal surface of the body is distinguished by two rows of 10 phosphorescent spots, one row on each side of the median line behind the carapace. Each spot is circular with irregular edges and is of a dirty yellow colour by daylight. The telson is a broad plate with a median emarginate tip (Fig. 1 T).

Appendages.—The first antenna extends ventrally beyond the carapace and is not hidden by it. The protopodite appears to be 3 jointed, cylindrical. The endopodite is short, slender and pointed—dactylose while the exopodite is two jointed, twice as long as the endopodite (Fig. 1 A). The second antenna is far longer, being nearly twice as long as the first. The three joints of the protopodite are cylindrical and the last two are marked by their distal edges being prolonged into a spine each (Fig. 1. B).

The mouth parts are attached medially just behind a rostral or labral plate which however does not form a complete sheath. The mandible, the 1st maxilla and the 2nd maxilla are attached closely together with their

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distal tips directed inwards and forwards. The mandible appears to be simple, blunt-tipped, and probably with a sharp posterior edge. The tips of the two mandibles are directed medially. The 1st maxilla is three-jointed, the basal joints are stout while the third is long, slender and pointed. The 2nd maxilla is two-jointed and tapers into a pointed process (Fig. 1 D).

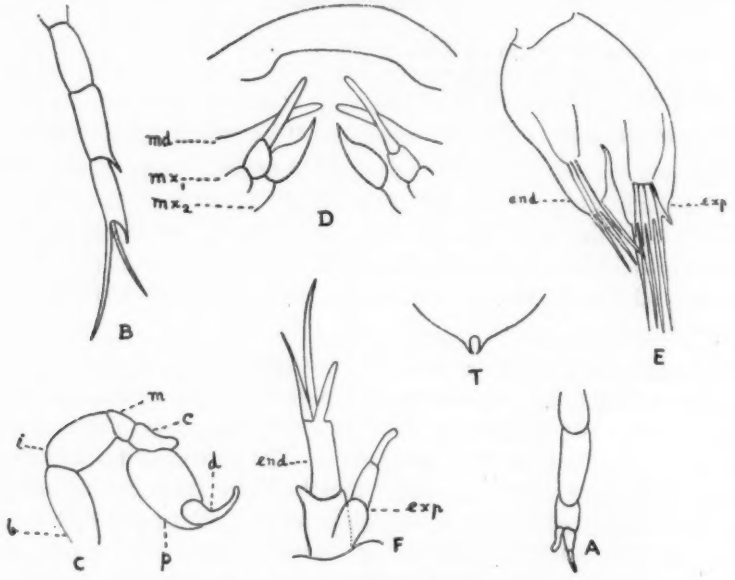

FIG. 1. *M. acartii* (Appendages)

As in all Isopods, there are seven pairs of six articled thoracic legs. The legs are short and prehensile. The proximal part curves outward, while the distal part, ending in a long, slender claw-like dactylus, is curved inwards (Fig. 1 C). This facilitates a purchase on the host's body. There are five pairs of biramous foliacious pleopods which are all alike and exhibit no trace of articulation. The exopodite of each bears a short, blunt spine at its outer distal edge and four setæ. The endopodite bears three setæ (Fig. 1 E). The uropod on either side is extended straight behind and is parallel and biramous. The protopodite is single-jointed bearing the endopodite on its inner side a little before its distal edge which is drawn out into a small spine on the outer side and bears the exopodite. The exopodite is long, cylindrical and bears three spines, the middle one being the longest. The endopodite is shorter and more slender. It is single-jointed and bears a spine (Fig. 1 F). The two uropods with their tapering spiny ramii are

pressed close together, to form the pointed, posterior end of the body and serve as a hold on the host.

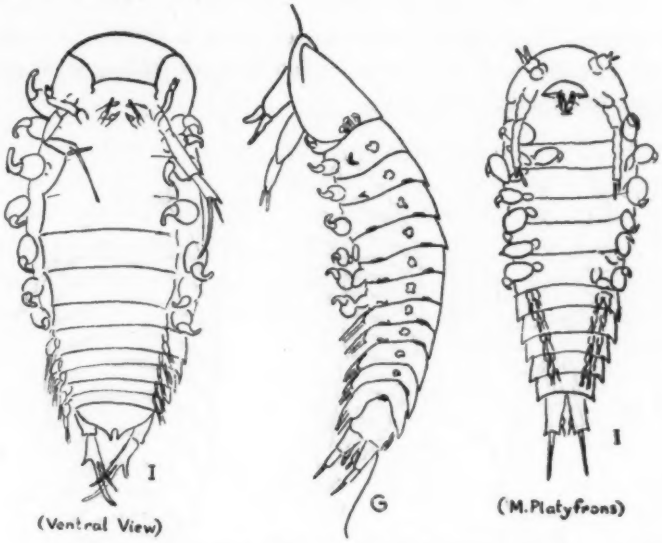

FIG. 2. Ventral and lateral views of *M. acartii* and ventral view of *M. platyfrons*

TYPE B

Microniscus eucalani

Host: *Eucalanus crassus* Griesbrecht.

Locality: Madras Coast.

Size of Host: 4·0 mm.

The parasite measured 1·32 mm. long and 0·48 mm. broad. The whole body is hirsute on the dorsal surface. There is no torsion of the body which is marked by a number of densely scattered red pigment spots giving the parasite a very light reddish colour. The edge of the cephalic plate is toothed and has no free flexible margin.

Appendages.—The 1st antenna is short, 3-jointed and uniramous. The basipodite has 3 spinous processes on its outer side. The 2nd joint is half as broad as the 1st and bears 3 setæ on its inner surface and a tuft of hairs on the outer side. The 3rd joint has 4 terminal bristles (Fig. 3 A). The 2nd (Fig. 3 B) antenna is nearly 5 times longer than the 1st. It is 8-jointed and flagellar. Each joint bears one or two spines on its distal edge while the terminal joint bears three long bristles.

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The mouth parts are similar to those of *M. acartii*, excepting for the difference in the lengths of the joints of the 2nd maxilla. In the present form all the mouth parts are directed anteriorly (Fig. 3 D).

The thoracic legs appear better fitted for prehension. The propodus is large and swollen, with the sharp-pointed dactylus folding on it giving the leg a chelate termination (Fig. 3 C).

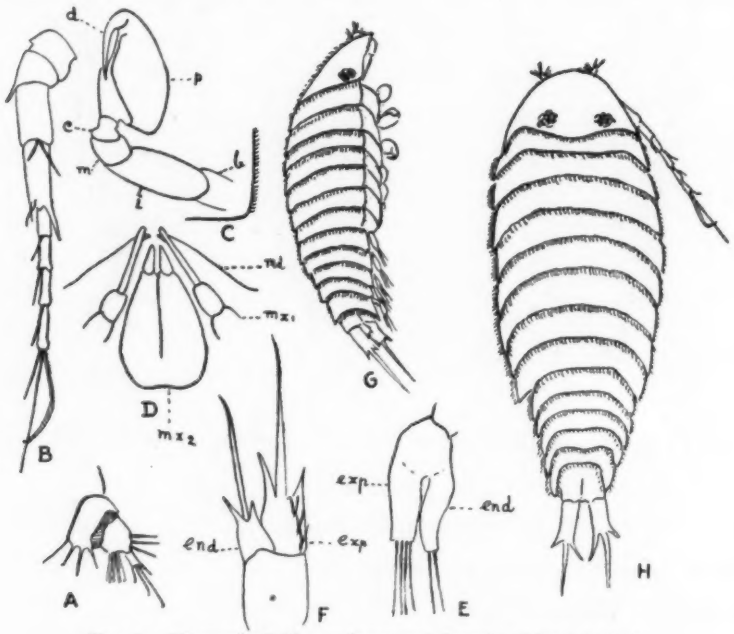

FIG. 3. *M. eucalanii* (Appendages and dorsal and lateral view)

There are five pairs of biramous pleopods. The endopodite bears two long setæ and the exopodite bears four long setæ (Fig. 3 E).

The uropods are biramous. The protopodite is single-jointed, stout, cylindrical. The exopodite has a sharp ridge bearing three slender spines and ends in a long curved spine.

The sides of the joint are produced into two stout, short spines, one on either side. The endopodite is smaller but single jointed. Its distal edge is drawn into two short stout spines, while it terminates in a long stout spine (Fig. 3 E).

Only one specimen of this type was found—though 20 samples each containing roughly 500 forms were examined.

TYPE C

Microniscus latyfrons

Hosts: *Acartis erythræa* and *Acrocalanus longicornis* Giesbrecht.

Locality: Madras Coast.

Size of Host: *Acrocalanus*: 1·42 mm. long. *Acartia*: 3 mm. long.

Out of the eight specimens collected, 6 were parasitic on *Acartia* and two on *Acrocalanus*. These measured 1·36 mm. long and 0·48 mm. broad. The carapace of this form differs in having the anterior region depressed so that the front edge is flatter.

Appendages.—The 1st antenna (Fig. 4 A) is biramous and short. The protopodite appears to be two-jointed, the proximal joint being very large

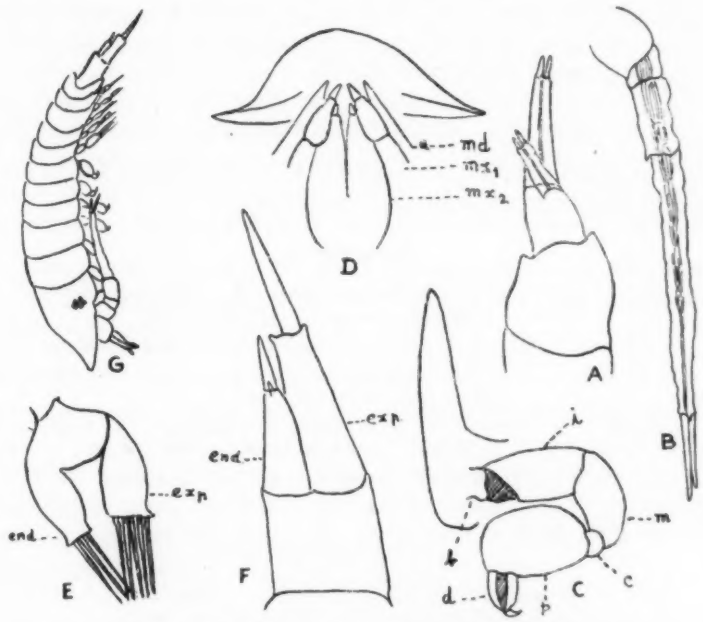

FIG. 4. *M. platyfrons* (Appendages and lateral view)

and stout. The second joint is about half its size, but longer and three times as stout as the endopodite. The exopodite is single jointed but twice as long as the endopodite. The endopodite is single-jointed, slender and ends in a pair of finger-like processes. The 2nd antenna (Fig. 4 B) is four-articled and three times as long as the 1st, uniformly thin and bears two small pointed processes at its tip.

The mouth parts (Fig. 4 D) are similar to those of the previous forms as regards their arrangement and structure. The difference in the lengths of the joints of the 1st maxilla and 2nd may however be mentioned.

There are seven pairs of six-jointed thoracic legs. The dactylus bears a small, curved pointed, process which helps in clinging. The propodus is large and rounded (Fig. 4 C).

There are five pairs of biramous pleopods. The expodite has a small spinous process at its outer edge and bears five setæ. The endopodite has three setæ (Fig 4 E).

There is a pair of biramous uropod. The protopodite is two-jointed, cylindrical. The endopodite is single jointed and bears a stout spine half as long as the base. The endopodite is distinguished by a small slender accessory spine. The exopodite also consists of a basal piece and a spine and the basal portion is as long as the entire endopodite and is proportionately stouter. The spine is also more than twice the length of the spine of the endopodite (Fig. 4 F).

Acknowledgement

We have great pleasure in thanking Dr. B. N. Chopra for having gone through the paper and made several suggestions.

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Key to Lettering

A.	1st antenna.	*M.*	Mandible.
B.	2nd antenna.	*Max.* 1.	1st Maxilla.
C.	Thoracic leg.	*Max.* 2.	Maxilla 2.
d.	Dactylus.	*E.*	Pleopod.
p.	Propodus.	*endp.*	Endopodite.
c.	Carpus.	*F.*	Uropod.
m.	Merus.	*exop.*	Exopodite.
i.	Ischium.	*endp.*	Endopodite.
b.	Basis.	*exop.*	Exopodite.
D.	Mouth-parts.	*T.*	Telson.

THE EFFECT OF ASPHYXIA ON THE MECHANICAL RESPONSE OF THE FROG'S UNSTRIATED MUSCLE AND ITS RELATION TO TONUS

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Received September 20, 1947

There are very few observations on record on the metabolism of frog's unstriated muscle, comparable to similar ones on the frog's heart (Clark, *et al.*, 1938); for reference to work on mammalian unstriated muscle, see Prasad (1935 *a*, *b*, 1936). Saiki (1908) found that there was very little glycogen in the frog's stomach and bladder and that the lactic acid content in fresh specimens was about 0·06 p.c. Lovatt Evans (1925) found that the lactic acid content of frog's stomach in the resting state was 0·03 p.c. and after stimulation at 20° C. at intervals of 45 sec. for 2 hours, it was 0·04 p.c.; this does not show any significant increase. The oxygen consumption of the frog's stomach muscle was found by Rao and Singh (1940) to be 0·015 c.c./g. per minute ($QO_2 \times 100 = 16 \cdot 2$); this is about the same as that for frog's heart (0·0133 c.c.; Clark, *et al.*, 1938). Stimulation with alternating current once every 15 min. doubled the oxygen consumption.

In the present research the effect of asphyxia on the mechanical response of the frog's stomach muscle was investigated; the chemical estimations will be reported in a later paper.

Experimental

In these experiments, unstriated muscle from the frog's stomach has been used (Singh, 1939). The cessation of oxygen supply to the muscle was produced by bubbling hydrogen. Either spontaneous activity was recorded, or the muscle was stimulated by alternating current, 8 volts/10 sec. per minute. It was also stimulated with potassium (0·02 M KCl), or by acetylcholine (1 in 10,000).

Results

The stimulant effect of asphyxia.—When asphyxiated or treated with sodium cyanide (1 in 10,000), frog's unstriated muscle differs from the frog's heart, in that the former shows a phase of increased excitability before final depression (Fig. 1). After the preliminary phase of increased excitability is over, the contractions may remain fairly constant for a long time (½ to 3 hours). Introduction of oxygen now causes a depression in excitability

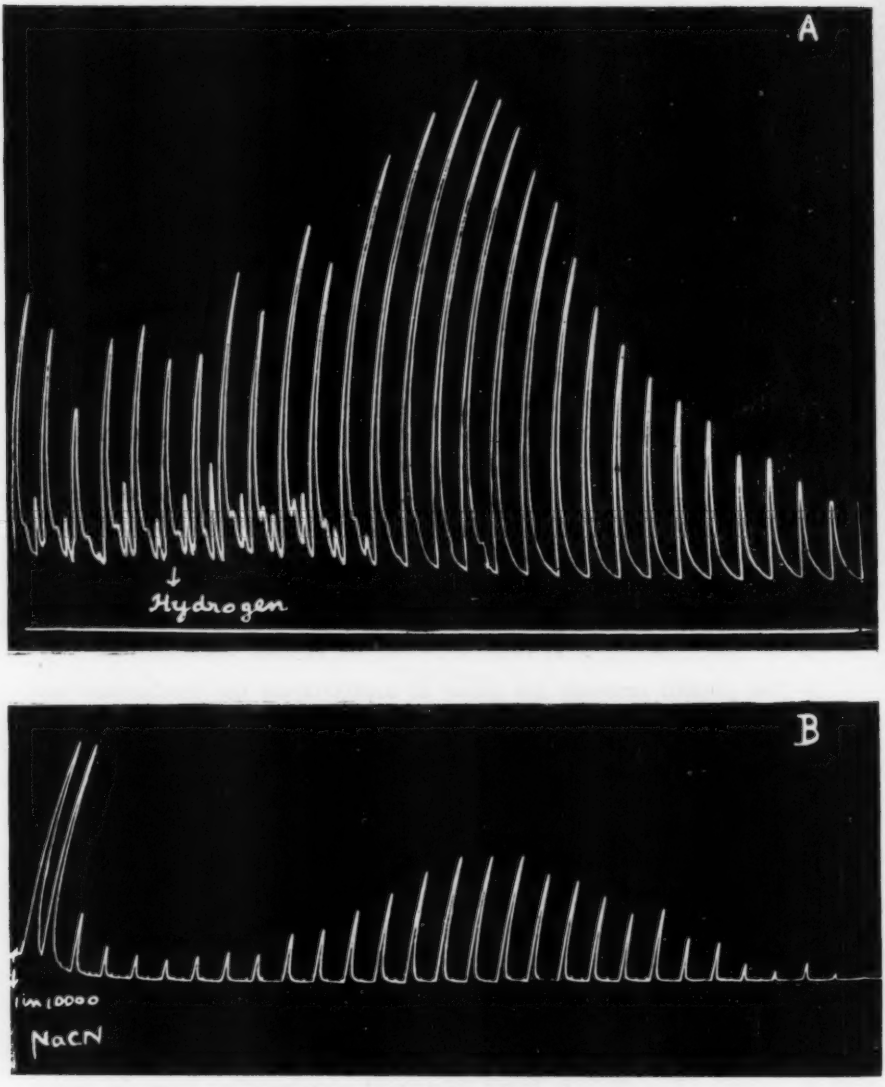

FIG. 1. Frog stomach muscle

A. Asphyxial increase of excitability to alternating current

B. Similar effect of NaCN, 1 in 10,000.

which may amount to complete paralysis (Fig. 2). This shows that in the muscle there are two mechanisms for obtaining energy, one ærobic and the

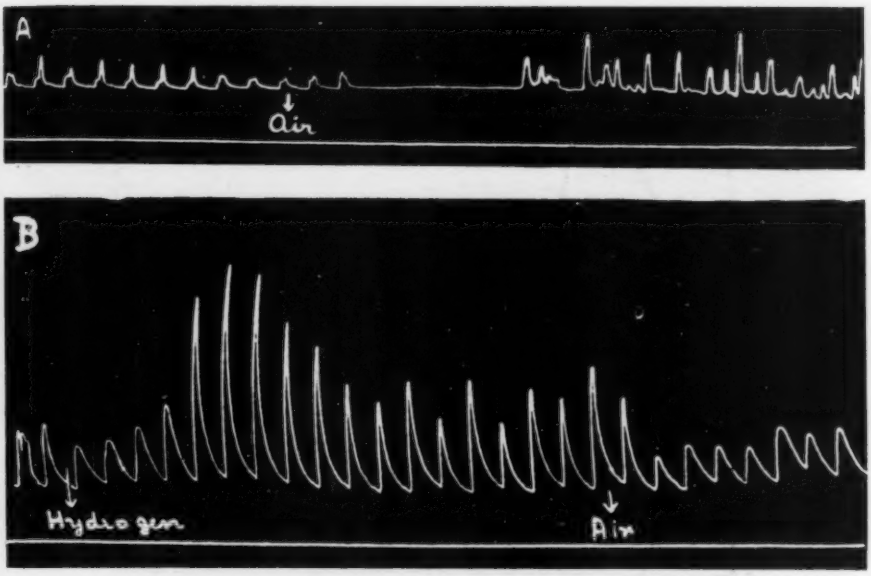

FIG. 2. Frog stomach muscle; stimulation by alternating current

A. Asphyxiated muscle. Complete paralysis on introduction of air followed by recovery

B. The effect of asphyxia and reintroduction of oxygen

other anærobic, which are mutually exclusive. These phenomena resemble those found in the chemoreceptors of the carotid body and in the nervous system. Thus the chemoreceptors of the carotid body are stimulated by oxygen lack and under certain conditions, their activity is depressed on the reintroduction of oxygen (Marshall and Rosenfeld, 1936). The stimulant effects of asphyxia in the central nervous system are well known (Lovatt Evans, 1945); reintroduction of oxygen may cause paralysis (Schmidt, 1941), Iodoacetic acid (1 in 20,000) does not abolish the asphyxial increase in excitability; this shows that it is not due to production of lactic acid. The optimum temperature for the increase in excitability is 30° C. (Fig. 3).

Relief of asphyxial arrest.—Asphyxial arrest is relieved by glucose in alkaline solutions and by substances that produce tonic contraction.

The stimulant action of glucose on the asphyxiated muscle is definite but it takes about 20–30 minutes for full development of the response, this presumably being the time required for diffusion of glucose. In the frog's heart, the action of glucose is more rapid, probably due to the fact that

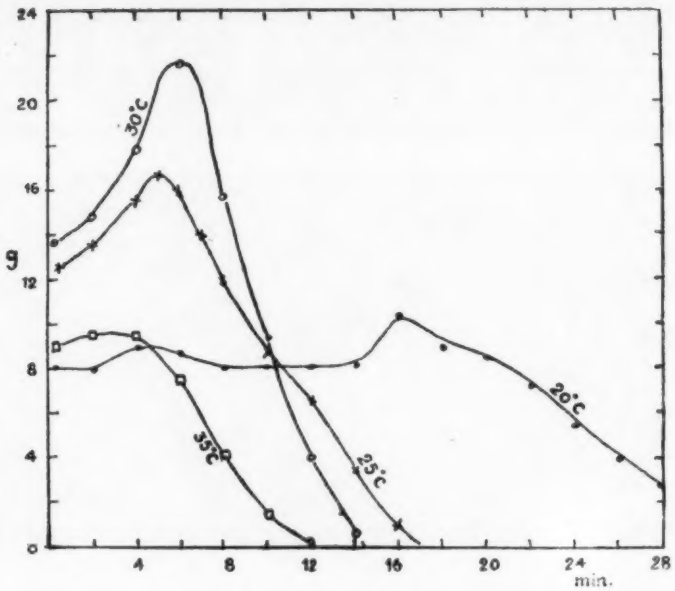

FIG. 3. Frog stomach muscle

Effect of temperature on the response of asphyxiated muscle to alternating current

diffusion is more rapid. As in the frog's heart, therefore, the stimulant action of any substance can be used to show whether it could be available as anærobic source of energy. The stimulant action must be permanent, as temporary changes lasting for 20–30 minutes might take place on introduction of any substance. The results are shown in Table I. The isolated muscle exhausted of carbohydrate by continued contraction in the absence of oxygen is revived by the addition to the perfusion fluid of glucose, but not of mannose, fructose, galactose, arabinose, xylose, maltose, lactose, sucrose, glycogen, starch, sodium lactate, glycine, asparagine, sodium acetate, sodium propionate, sodium butyrate, sodium oleate. Frog's unstriated muscle, thus differs from the frog's heart in that it cannot utilise mannose.

Other substances that relieve asphyxial arrest are those which produce tonic contraction, such as cations, Li, NH_4, K, Ba; anions, NO_3 I, SCN; drugs, eserine, acetylcholine. The power of these substances to relieve asphyxial arrest is proportional to their power in producing tonic contrac-

TABLE I

Beneficial action of carbohydrates, etc., on (a) asphyxiated and exhausted frog's stomach muscle and (b) the asphyxiated and exhausted frog's heart

Subject	(*a*) Asphyxiated and exhausted frog's stomach muscle	(*b*) Asphyxiated and exhausted frog's heart (Gaddie and Stewart, 1934)
Polysaccharides :		
Starch ..	−	−
Glycogen ..	−	−
Disaccharides :		
Maltose ..	−	−
Lactose ..	−	−
Sucrose ..	−	−
Monosaccharides :		
Glucose ..	+	+
Mannose ..	−	+
Fructose ..	−	−
Calactose ..	−	−
Pentoses :		
Arabinose ..	−	−
Xylose ..	−	-
Amino acids :		
Glycine ..	−	−
Lipoids :		
Sodium oleate ..	−	−

tion; potassium is the most powerful (Fig. 4). In contrast to the action of glucose, these substances are more effective in acid than in alkaline solutions, even at pH 6, and in the presence of iodoacetic acid. These experiments show that twitch and tonic contractions, besides differing from each other in several respects, as described in previous papers, also differ as regards their metabolism.

Effect of asphyxia on tonus.— Asphyxia has two effects on tone and tonic contractions mentioned above. At first tone decreases and then increases (Fig. 5). This asphyxial contraction can be maintained for several hours and is easily reversible, though after 12 hours, it becomes irreversible, and the condition resembles *rigor mortis* of striated muscle. These findings are in agreement with the oxygen consumption measurements (Rao and Singh, 1940). It was found that two kinds of tonic contractions were produced; during one the oxygen consumption increased, and during the other, it decreased.

It has been shown that substances that produce tonic contraction, retard the relaxation of unstriated muscle (Singh, 1938); in confirmity with this,

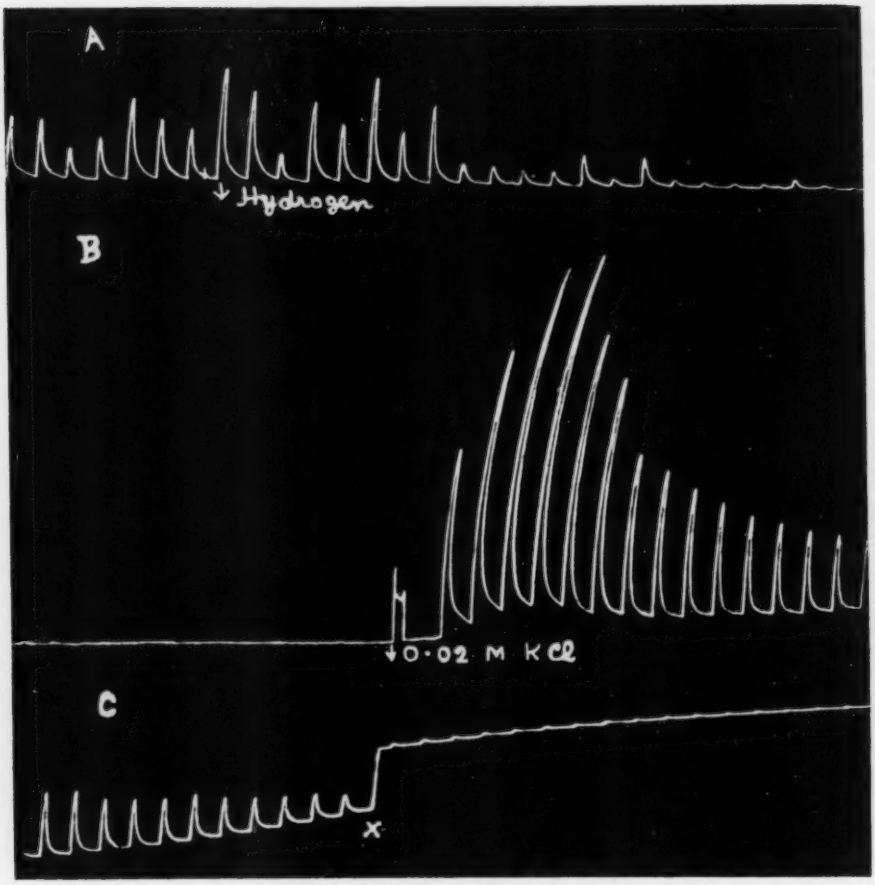

FIG. 4. Frog stomach muscle, pH 6

A. Asphyxial arrest by hydrogen
B. Relief of asphyxial arrest by potassium
C. Above tracing continued. Drum stopped for 15 minutes at ×. Note asphyxial contraction

it has been found that relaxation may be retarded as a result of asphyxia. Cyanide retards relaxation (Rao and Singh, 1940).

Action of iodoacetic acid and acid solutions.—The fact that the asphyxial exaltation of excitability is not suppressed by iodoacetic acid, causes frog's unstriated muscle to behave quite differently from frog's heart and mammalian unstriated muscle when deprived of oxygen, its movements being not rapidly

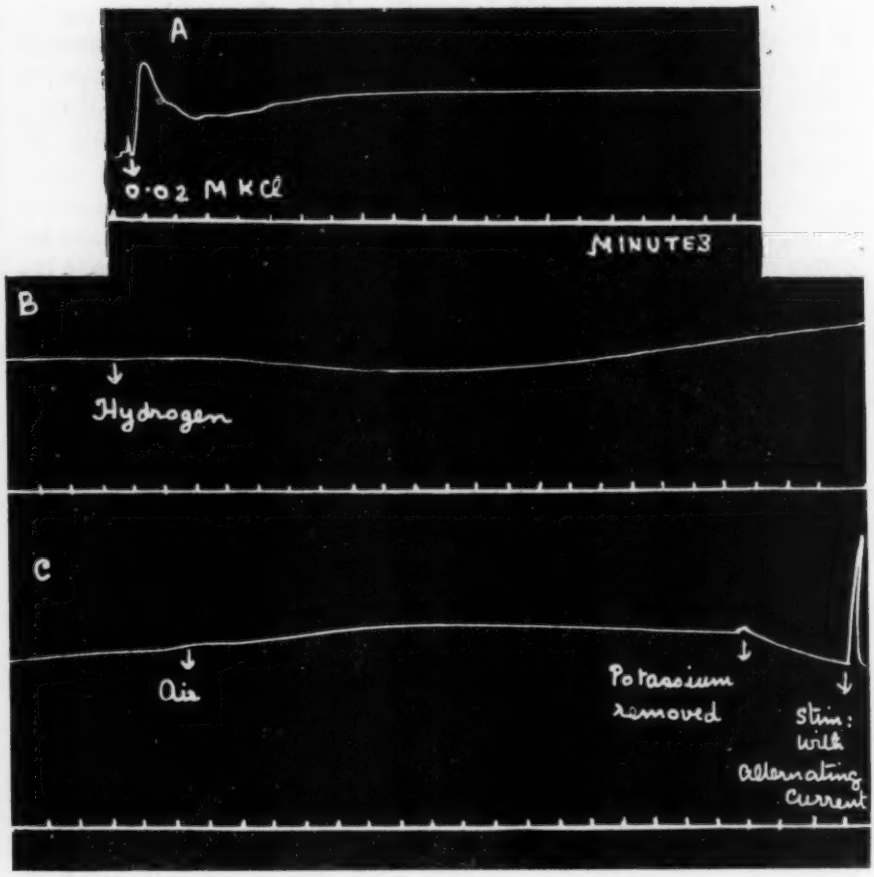

FIG. 5. Frog stomach muscle

A. Stimulation by 0·02 M KCl

B. When the response is steady hydrogen is introduced

C. Introduction of air. After a few minutes tone begins to decrease. On removal of potassium, tone decreases and the muscle responds normally to alternating current

arrested. In a muscle poisoned with iodoacetic acid, asphyxia causes rapid arrest if the pH is raised to 6, as at this reaction, the asphyxial increase in excitability does not occur. These experiments show that though both iodoacetic acid and pH 6 suppress production of lactic acid, their actions are not identical.

If a muscle, poisoned with iodoacetic acid, is exhausted by stimulation in the absence of oxygen and then revived by oxygen, the response is never as big as before. As in the case of frog's heart, the response is improved by certain substances, which are sodium lactate and sodium butyrate. Glucose, glycine, sodium acetate and sodium propionate do not improve the response.

In the presence of oxygen, mammalian unstriate muscle becomes hyper-excitable in acid solution (Singh, 1940). Frog's unstriated muscle also becomes more sensitive in acid solutions. This suggests that the muscle has developed some other ærobic mechanism to deal with acid solutions, as the production of lactic acid suppressed by such solutions.

The effect of substances mentioned in connexion with the action of glucose, was also studied in an exhausted muscle at pH 6 in the presence of oxygen. It might be of significance in metabolism of the muscle in acid solutions. Glucose, mannose, fructose, galactose, sucrose, lactose, maltose, glycogen and starch had no effect, nor did glycine have any beneficial action. Sodium acetate, propionate improved the response, but not sodium butyrate or oleate. Sodium lactate improved the response.

Discussion

Frog's unstriated muscle differs from the frog's heart in many respects. It has several ærobic and anærobic metabolic mechanisms. Thus it has a different ærobic mechanism to deal with acid solutions. It has an anærobic mechanism, other than the glycogen-lactic acid system, and it has a separate mechanism for tonic contractions.

The fact that potassium releases some store of energy in a muscle poisoned with iodoacetic acid, may be of importance in the process of stimulation by electric current; the latter would cause movement of potassium ions inside the fibres and increase its concentration at some membrane. This would release the energy for contraction.

A new theory of tonus.—In asphyxial contraction is a mechanism whereby the muscle can maintain tension without expenditure of energy. It is therefore, reasonable to assume that tonus of muscle which requires no extra energy for maintenance would be identical with or related to the asphyxial contraction. The part of tonic contraction which does not require energy for maintenance is the slow relaxation; as slow relaxation occurs as a result of asphyxia or cyanide, the asphyxial contraction appears to be related to tonus that does not cause increase in oxygen usage. This is further supported by the fact that the oxygen consumption may diminish during

normal tonic contraction (Lovatt Evans; Rao and Singh) and increase during inhibition (Rao and Singh).

The asphyxial contraction suggests that the normal state of the myosin molecule is that of shortening. This would account for the shortening of muscle without increased oxygen consumption. How can, then, the other kind of shortening, which is accomopanied by increase in oxygen consumption be accounted for? Our previous scheme (Singh and Singh, 1946) has to be amplified.

If MT, which is a compound of myosin (M) and another chemical substance T, represents the shortened molecule, then normally the effect of T is neutralised by some other substance C. MTC, then, may represent myosin in its relaxed state. Postulation of T however is not necessary; the relaxed molecule may be represented by MC. Contraction, then, can be caused by two processes: (i) If the influence of C upon M is removed; (ii) or if another substance combines with MC, say Q, which forms MCQ; in the first instance MC may be dissociated or split by some other substance N.

$$CM + N = C + MN.$$

The maintenance of tension in this case will not require expenditure of energy. In the second instance, the compound MCQ or Q itself may be unstable, and so require continuous expenditure of energy. The tonic and twitch contractions will then be respectively represented by M and MCQ.

Summary and Conclusions

1. Asphyxia at first increases the response of frog's unstriated muscle; this is followed by diminution and then paralysis. These effects are also produced by cyanide. This increase in excitability is not abolished by iodoacetic acid.

2. Asphyxial arrest is relieved by glucose, potassium and the other substances that produce tonic contraction.

3. In the presence of oxygen the muscle becomes hyperirritable in acid solutions and so possesses different ærobic metabolic mechanisms in alkaline and acid solutions respectively.

4. In acid solutions, pH 6, sodium lactate, acetate and propionate improve the response to alternating current.

5. Asphyxia does not produce rapid arrest of movement in muscle poisoned with iodoacetic acid.

6. In a muscle poisoned with iodoacetic acid, then exhausted by frequent stimulation in asphyixia, and then revived by oxygen, sodium lactate

and sodium butyrate improve the response. Glucose, glycine, sodium acetate and propionate have no effect.

7. In asphyxia, tone at first decreases and then increases; this asphyxial contraction is identical with tone that does not use oxygen.

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STUDIES ON THE EMBRYOLOGY OF MICROCHIROPTERA

Part II. Reproduction in the Male Vespertilionid Bat *Scotophilus wroughtoni* (Thomas)

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Introduction

A study of the reproductive phenomena in bats offers a number of interesting features. In this paper an attempt is made to record the cyclical changes in the male reproductive organs in the breeding and the non-breeding seasons. As mentioned in the first part of this series (1947)[3] the study is based entirely on the examination of the wild specimens for the obvious reason that any experimental study is impossible in the caged bats. The specimens were all collected round about Bangalore, which is a tropical zone of more or less unvarying climatic conditions throughout the year. A general account of the breeding seasons of this species of bats has formed the subject-matter of the first part of this paper. It was based upon a gross study of the females only. The results of the study of the male reproductive organs are incorporated in this paper; and these confirm the conclusions already arrived at regarding the breeding seasons.

Historical

Though a large volume of literature is available on the general breeding habits of the insectivorous bats, and also on the female sexual cycle of many species, it is a matter of considerable surprise that very little work has been done on the sex-cycle of the male bats. The litle information we have on the subject of the reproductive processes of the male bats is the result of a study of the female genitalia for the presence of spermatozoa in the vaginal tract or the oviduct and the casual observations on the exaggerated secondary sexual characters in the male during the breeding season.

A study of the literature on bat reproduction reveals that in a majority of the species inhabiting the temperate and cold climates, the males experience a height of sexual activity during late autumn when they copulate, followed by hibernation during winter. The spermatozoa also undergo hibernation in the genital tract of the female and fertilize the ovum in the next spring. A large volume of circumstantial evidence has also accumulated to substantiate this view. Courrier (1927)[2] has shown in *Pipistrellus pipistrellus* that the examination of the male genitalia revealed the fact that after copulation

in late autumn the testes degenerated and throughout winter contained no spermatozoa, but only spermatogonia and sertoli cells. Nakano (1928)[8] working on *Vespertilio abramus* has come to similar conclusion that after copulation in October the testes degenerate. A very positive proof of autumn copulation was given by Harrison Matthews (1937)[5] working on the British Horse-shoe Bats. Because of the occurrence of a hard vaginal plug in the female throughout the winter after copulation he concludes "The ovum must have therefore been fertilized by one of the spermatozoa stored in the upper part of the genital tract and the spermatozoa must have been deposited in the previous autumn."

On the other hand, there are a few species of insectivorous bats in which copulation occurs in spring followed immediately by ovulation and fertilization resulting in pregnancy.

Baker and Bird (1936)[1] recorded in *Miniopterus australis* which is a tropical species that there is an annual sexual rhythm in the males which show sexual activity in July, August, September, and October (*i.e.*, the southern spring), and the testes would be nonfunctional during the rest of the year. They recorded as follows: "Looking at the material as a whole one sees clearly that copulation takes place about the end of August, and that the development of the embryo starts at once. Copulation occurs at a time of the year when the days begin to get longer and the temperature is rising." Recently, Harrison Matthews (1942)[6] recorded his observations on some of the South African bats in which copulation occurs in spring and is immediately followed by fertilization and pregnancy.

There is still a third kind of observation. Rolland, E. Miller (1939)[7] working on *Myotis lucifugus lucifugus* and *M. greisesens* observed that "Copulation is known to occur in the fall. The conditions of the accessory glands and the presence of sperms make recurrent copulations possible until spring." On examining the accessory reproductive glands he recognised that the glands were functional and the epididymis full of sperms throughout winter and spring. He was convinced that there was a period of spring copulation too. He is, however, not certain whether the ovum is fertilized by the sperm of spring copulation or fall copulation. Mary, J. Guthrie (1933)[4] recorded that spring copulation is a normal occurrence in some American cave bats. She believed that the spermatozoa stored in autumn copulation are destroyed by the numerous phagocytes of the genital tract and copulation occurs again in early spring.

It is evident from the foregoing summary that most of the work relates to bats of the temperate regions in which two very different types of observations are recorded. There is practically no work on tropical bats except

that of Baker and Bird (1936)[1] and recently of Harrison Matthews (1942).[6] Both these authors observe that copulation takes place in spring and is immediately followed by fertilization and pregnancy.

Material and Methods

Bats were collected from the hollows of the trees in forests round about Bangalore. They were killed by chloroform and immediately dissected. Unfortunately their body weights were not taken. The genital organs, kidneys and suprarenals were fixed. Bouin's picro-formal-acetic was the usual fixative used. But other fixatives like Sanfelice's formula and corrossive acetic were also used. The weight of the testis and the epididymis was taken together after preservation in 70% alcohol. They were sectioned and stained in hæmetoxylin. The measurements of the diameters of the testis tubules and the tubules of the epididymis were taken by the use of a micrometer scale.

Table I gives the data of collections of the males and females. I began collections in May 1945 and it is still in progress.

TABLE I

Monthly record of collections: *Scotophilus wroughtoni* (Thomas)

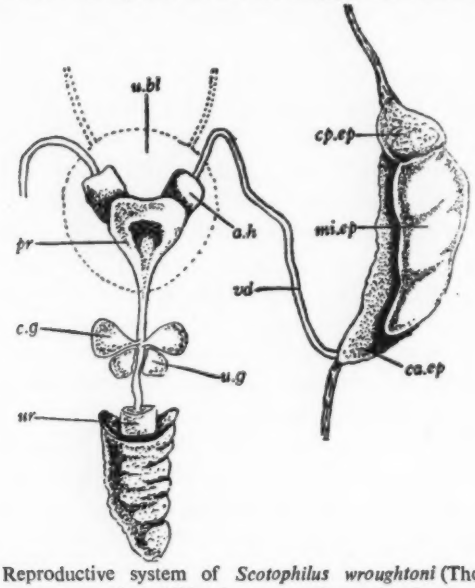
Month	Date	Males	Females
May ..	17—5—1945	2	28
,, ..	7—5—1946	2	2
June ..	7—6—1946	2	2
July ..	..	No collections	
August ..	..	do	
September ..	18—9—1946	1	1
October ..	20-10—1945	1	1
November ..	8-11—1945	2	2
December ..	7-12—1946	2	17
January ..	8—1—1947	3	5
February ..	10—2—1946	5	3
March ..	22—3—1946	2	4
April ..	1—4—1946	1	6
,, ..	22—4—1946	4	26
,, ..	28—4—1947	1	9
,, ..	29—4—1946	1	20
Total ..		29	126

The numbers do not give any idea of the sex-ratio or the population of these animals, primarily because of the small number of specimens collected and secondly because of the probability that the males and the females must have lived separately after the active breeding period. It is worth noticing, however, that during January and February the proportion of males to females is markedly higher than during the other months. This may probably roughly indicate that during this period the males and the females live together.

Observations

(A) *The Male Reproductive Organs* (Text-Fig. 1)

The male reproductive system consists of a pair of testes whose position and size varies during the different seasons of the year. The testes are abdominal in position during the non-breeding periods and becomes inguinal



Text-Fig. 1. Reproductive system of *Scotophilus wroughtoni* (Thomas) × circa 6. *a.h.* ampulla of Henle ; *ca. ep.*, cauda epididymis ; *c. g.*, Cowper's gland ; *cp. ep.*, caput epididymis ; *mi. ep.*, mid-epididymis ; *pr.*, prostate ; *u. bl.*, urinary bladder ; *u. g.*, urethral gland; *ur.*, urethra ; *vd.*, vas deferens.

and sometimes even post-anal at the height of sexual activity, consequent not only upon their migration but also upon their enlargement. Each testis is slightly tapering towards the caudal end.

The epididymis is not very clearly demarked into a caput (*cp. ep.*), a mid (*mi. ep.*), and a cauda (*ca. ep.*) epididymis. The names are given to the portions of the epididymis in the figure by reason of the position. But on the onset of the breeding season the cauda epididymis (*ca. ep.*) is much elongated. The vas deferens (*vd.*) arises from the cauda epididymis (*ca. ep.*) and passes upwards on the median side of the testes between it and the bladder (*u. bl.*). Each vas deferens at its distal end is swollen up into a bean-shaped structure—the ampulla of Henle (*a.h.*). A true seminal vesicle distinct from the ampulla of Henle is absent. Thus the structures bear resemblance to some of the South African bats described by Harrison Matthews (1942).[6] The ampullæ of Henle are embedded in the wall of the

prostate glands (*pr.*) for nearly two-thirds of their length as in *Miniopterus minor* and *M. dasythrix* (Harrison Matthews, 1942).[6] The prostate is a fairly large structure lying below the bladder. The urethra (*ur.*) a little down below the prostate is joined by a pair of small pear-shaped glands—the Cowper's glands (*c. g.*). These lie just above the rectum. Posterior to the Cowper's glands are a pair of urethral glands (*u. g.*). The penis is directed caudally.

(B) *Seasonal Variations in Testis*

(i) *Macroscopic Examination*

Each testis was weighed along with its epididymis. Table II gives these weights for the several months of the year. It is a well-known fact that in

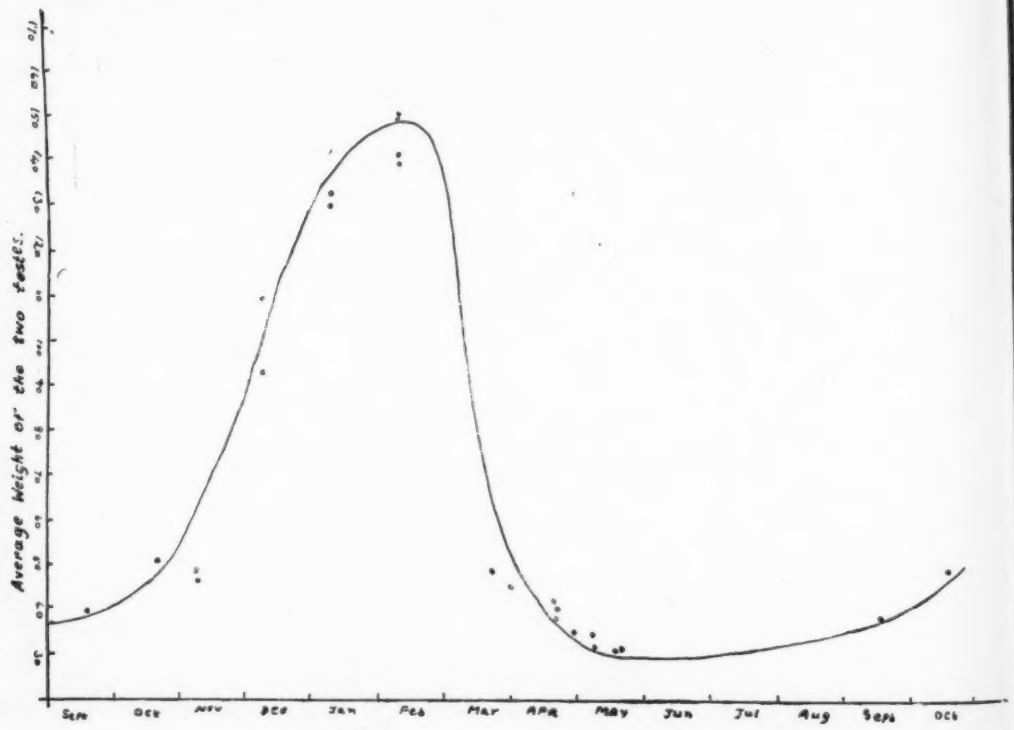
TABLE II

Scotophilus wroughtoni (Thomas)—*Macroscopic and Microscopic Measurements of the Testes and the Epididymides*

Month	Date	Index No.	Weight of the testes and epididymides in milligrammes				Diameters of the seminiferous tubules (in microns)			Diameters of the epididymal tubules (in microns)		
			Right	Left	Total	Average	Smallest	Largest	Average of 10	Smallest	Largest	Average of 10
January ..	8—1—47	123	145	142	287	143·5	156	170	160	93	114	101
do	do	128	130	136	266	133·0	..	..	..	..	..	..
do	do	129	132	128	260	130·0	140	170	151	107	114	108
February ..	10—2—46	32(i)	143	140	283	141·5	..	..	..	..	..	..
do	do	32(v)	..	..	..	..	..	..	..	..	..	..
do	do	32(vi)	150	152	302	151·0	171	189	178	107	128	120
do	do	32(vii)	135	144	279	139·5	167	186	174	110	128	118
do	do	32(viii)	148	153	301	150·5	..	..	..	..	..	..
March ..	22—3—46	33(*c*)	48	51	99	49·5	..	..	..	..	..	..
do	do	33(*d*)	52	53	105	52·5	78	111	101	70	104	78
April ..	1—4—46	45	46	46	92	46·0	..	..	..	..	..	..
do	22—4—46	57	42	40	82	41·0	68	93	87	54	68	62
do	do	63	40	42	82	41·0	..	..	..	..	..	..
do	do	64	38	47	85	42·5	..	..	..	..	..	..
do	do	66	40	36	76	38·0	..	..	..	..	..	..
do	28—4—47	138	36	38	74	37·0	..	..	..	..	..	..
do	29—4—46	88	38	33	71	35·5	..	..	..	..	..	..
May ..	7—5—46	100	32	33	65	32·5	57	86	69	29	50	38
do	do	101	36	..	..	36·0	..	..	..	..	..	..
do	17—5—45	9	34	30	64	32·0	..	..	..	..	..	..
do	do	27	33	29	62	31·0	53	78	64	29	48	38
June ..	7—6—46	108(*a*)	..	..	..	..	..	..	..	..	..	..
do	do	111(*f*)	..	..	..	..	61	86	68	24	41	35
July August	No specimens were available and all the expeditions for collections were fruitless						..	..	..	..	..	..
September	18—9—46	113(*a*)	38	41	79	39·5	71	93	80	43	60	56
October	20—10—45	30(*a*)	49	51	100	50·0	73	106	96	50	73	61
November	8—11—45	31(*c*)	47	50	97	48·5	..	..	..	..	..	..
do	do	31(*d*)	46	46	92	46·0	114	143	120	64	93	81
December	7—12—46	106(*a*)	90	96	186	93·0	134	161	150	93	114	100
do	do	118(*b*)	107	112	219	109·5	121	157	144	71	100	92

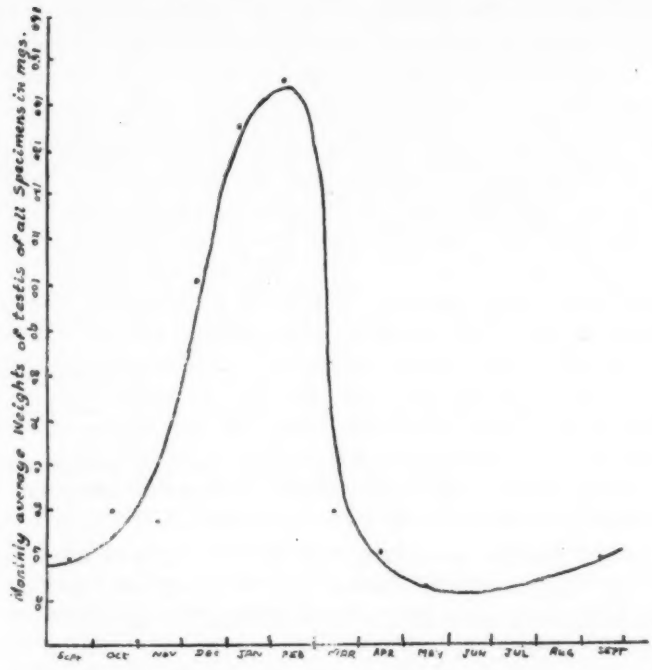
the case of the insectivorous bats the growth of the young is very rapid and furthermore, unlike the bats of the temperate and cold climates all the males were fecund during the breeding seasons.

Text-Fig. 2 is a graph of the average weights of the testis and epididymides of the two sides plotted against the months. Text Fig. 3 gives the graph of the weights of the testes average of all specimens of the month plotted against the months.



TEXT-FIG. 2. Graph to indicate the increase in the weight of the testis during the breeding season. The average weight in milligrammes of the two testes of each specimen is plotted against the different months of the year. There is an enormous increase in the weight of the testes during the months of January and February.

The graphs clearly indicate that the weight of the testis increases rapidly during the months of December, January and February, and suddenly decreases during the month of March, after copulation, and the following months till September. The weight begins to increase from October onwards which marks the onset of sexual activity. The February testis is nearly four times as heavy as the testis of September.

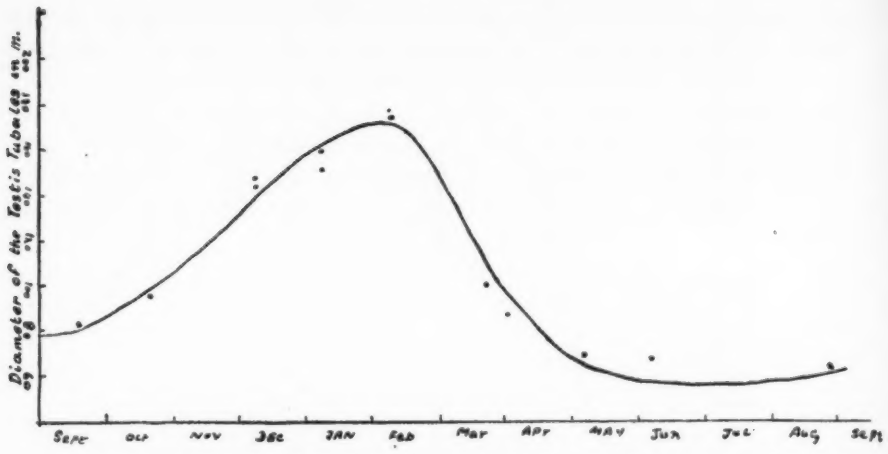


TEXT-FIG. 3. Graph of the average weight of the testes of all specimens of each collection plotted against the months.

(ii) *Microscopic Examination*

(*a*) *Diameter of the seminiferous tubules.*—The size of the seminiferous tubules varies during the several months of the year according to the activity of the testis. Table II gives the diameters of the seminiferous tubules during the different months of the year. For the sake of convenience the measurements of the seminiferous tubules of only one specimen from the collection of each month is taken. There is no marked difference among the testis tubules of the several specimens of any one collection. Diameters of ten tubules are taken, five situated near the periphery and five near the centre and a mean value is arrived at. Text-Fig. 4 is a graph representing the variation in the diameter of the seminiferous tubules from the specimens collected during the different months of the year.

The mean diameter which was only 80 μ in September suddenly increases on the onset of the activity of the germinal epithelium and increases to 150 μ during December and January, and reaches a maximum diameter of 180 μ



TEXT-FIG. 4. Graph to indicate the increase in the diameter of the tubules of the testes during the breeding season. There is a considerable increase in the diameter of the tubules during the months of January and February. The diameters are taken in microns.

in February (10–2–46). As already remarked the testes of the several specimens of any one collection show similar seminiferous tubules. It is an important fact to note as it gives us a possible age of the specimens at which the male bats become sexually mature.

During March there is a sudden decrease in the diameter of the seminiferous tubules, and the tubules of May specimens show a small diameter of 60 μ to 70 μ, and very much the same as the September testis.

The diameter of the testis tubules seems steadily to increase with the weight of the testis. Text-Fig. 6 is a graph to show the relationship of the diameter of the testis tubules and the weight of the testis.

(*b*) *Histological changes in the testis.*—The microscopic examination of the sections of the testis indicates that in the specimens collected between the months of April to November, the tubules are inactive showing the germinal epithelium cells with resting nuclei. The tubules have no lumina, and the intercellular spaces are large. But in specimens collected in December the tubules have sprung up to activity showing plenty of mitotic figures. The spermatogenetic activity reaches its maximum vigour during January and February.

In May the testis tubules are small and have no lumina (Plate IV, Fig. 1). The tubules are composed of resting spermatogonia and sertoli cells. The nuclei of the spermatogonia are large, spherical and lightly stained and the individual cells are clearly marked out. The sertoli cells

are not so distinctly marked out. Their nuclei are small, are more densely stained, and they appear to be floating in the sertoli syncytium.

The sections do not show the presence of many capillaries.

The condition of the testis remains more or less the same till September. The testis tubules are still without lumina, and the sertoli cells are small and darkly stained. There are two or three layers of spermatogonia at the periphery with large spherical nuclei. Some spermatogonia are undergoing division. The intertubular spaces are still large. The vascularisation of the testis is still low.

The testes of November collection show lumina in a few of the peripheral tubules, but the tubules in the centre are still without lumina. The intertubular area is reduced. The tubules contain mainly the darkly staining spermatocytes and the sertoli cells, which are few in number.

In December the testis tubules have greatly enlarged and most of the tubules have clear lumina. The germinal epithelium is active showing all stages of spermatogenesis (Plate V, Fig. 3). The peripheral part of the tubule contains a few spermatogonia with large faint nuclei abutted against the wall of the tubule and very few in number. But the majority of the cells are spermatocytes in various stages of spermatogenesis. Towards the central part of the tubule there are a large number of spermatid nuclei, which are characterised not only by their small size but also by their darkly staining nuclei. A few of the spermatids have undergone spermateleosis and consequently the lumen presents even ripe spermatozoa. The sertoli cells are reduced in number.

In January and February the testis is in full swing of activity with large numbers of meiotic nuclei (Plate V, Fig. 4 and Plate VI, Fig. 6). The lumen of the tubule is enlarged and is packed with spermatozoa. All the sper matids are undergoing the final change and large swarms of sperms are found radiating towards the centre.

As the tubules have greatly hypertrophied the intertubular space is reduced to a minimum.

The vascularisation of the testis is markedly high and a number of capillaries are seen in the sections, both in the mass of the testis and also in the walls.

(C) *Seasonal Variations in the Epididymis*

The epididymis also shows a seasonal variation corresponding to the variation in the testis. Table II gives the data regarding the variation in the diameter of the epididymal tubules during the different months of the year.

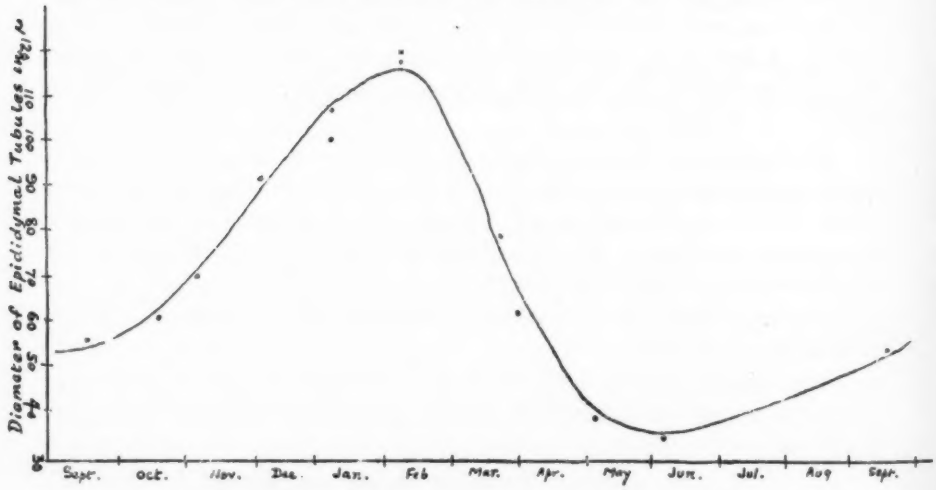
The tubules which were very narrow during the months between May and September, widen out from November onwards. The epithelium which appears to be two or three layered (due probably to shrinkage) flattens out into a single layered cubical epithelium.

From May to September there does not seem to be any change in the diameter of the tubules. It is narrow and varies from 29 μ to 43 μ in May to 43 μ to 60 μ in September. The intertubular area is large and is traversed by connective tissue and circular muscles (Plate IV, Fig. 2).

During November the diameter of the tubules is already large (57 to 64 μ). And in December the diameter increases to 100 μ to 114 μ and the intertubular spaces are much reduced. Furthermore the lumina contain spermatozoa.

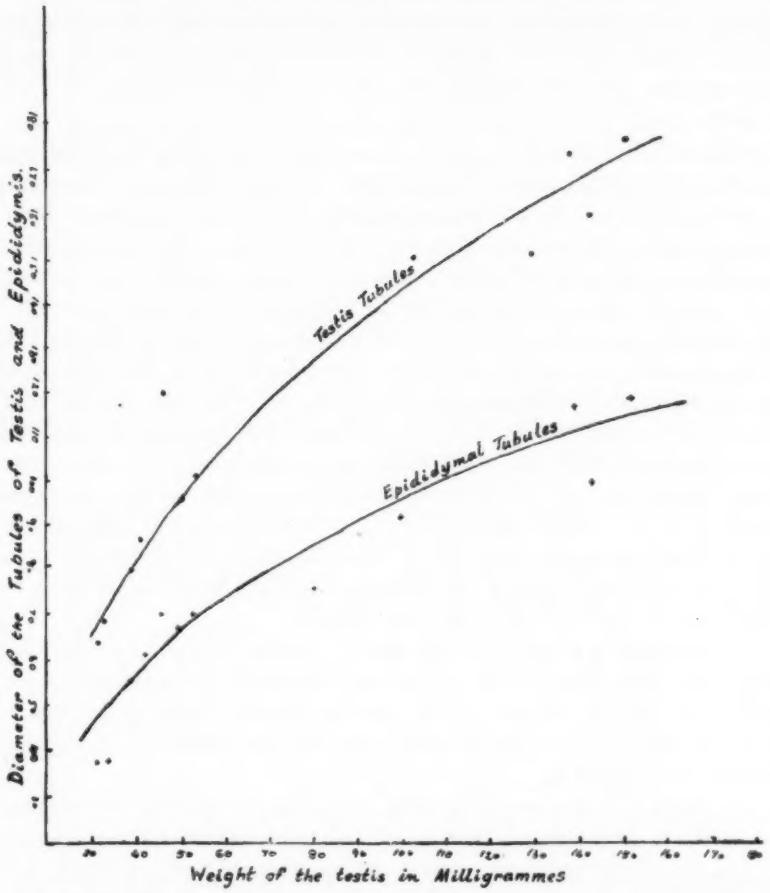
During January and February the diameter of the epididymal tubules reaches a maximum of 114 μ to 128 μ and all the lumina are packed with spermatozoa. The intertubular space is negligible (Plate VI, Fig. 5 and Plate VII, Fig 7).

Text-Fig. 5 is a graph to indicate the increase in the diameter of the epididymal tubules during December, January and February, and its comparatively narrow condition during the rest of the year.



TEXT-FIG. 5. Graph to indicate the increase in the dameter of the tubules of epi li-dymis during the breeding season. The diameters are the largest between the months of January to March. The diameters are measured in microns.

Text-Fig. 6 shows the relationship that exists between the increase in the diameter of the tubules of the epididymis and the increase of the weight of the testis.


Diameter of the Tubules of Testis and Epididymis.
Testis Tubules
Epididymal Tubules
Weight of the testis in Milligrammes

TEXT-FIG. 6. The diameters of the tubules of the testes and of epididymis plotted against the weights of the testes. The graph is a rising curve indicating that the diameters increase with the weight of the testes. The upper graph indicates the relation between the weight of the testes and the diameters of the testes tubules and the lower graph shows the relation between the weight of the testes and the diameter of the tubules of the epididymis.

The figures clearly show that the increase in the diameter of the epididymal tubules runs parallel to the increase in the weight of the testis, which in its turn is directly related to the functional activity of the testis.

CONCLUSIONS

1. *The Breeding Season.*—The progressive increase in the weight of the testes during the months from December to January, and its weight being at its lowest during the other months of the year taken along with the fact

that the testis contains the spermatozoa only during these months, indicates that the height of sexual activity is reached in these animals during these three months and the animals are sexually quiescent during the rest of the year.

The examination of the males alone cannot give us any definite proof regarding the exact period of copulation. The occurrence of large swarms of spermatozoa in the epididymis during January and February indicates the probability of copulation during these months. But the examination of the female genitalia (Gopalakrishna, 1947)[3] has revealed that the females have not only not ovulated on the 10th February but their genetalia contained no spermatozoa. Thus copulation had not occurred till the 10th of February. The conclusion therefore seems to be that the males become sexually active and functional nearly 6 to 8 weeks before the females ovulate. Further during the months of July and August the females are all lactating, and there is no evidence to show that lactating females become pregnant. Hence the absence of collection during July and August does not seriously impede us from concluding, without any possibility of error, that the males like the females experience an annual breeding cycle, and this roughly corresponds to the same months as the females, the males becoming sexually active a little (6 to 8 weeks) earlier than the females.

After copulation which I have shown to occur between the 10th February and the 22nd March (1947 *a*), the males probably live separately from the females, for during the rest of the summer though many expeditions were made and large number of specimens collected, the number pf males captured was very low (Table I).

2. *Age of Maturity*.—A second important conclusion we may arrive at from the available data is that, as all the males collected during December, January and February were sexually active, animals born at about the end of June must have a rapid growth and must become sexually mature during the next January, *i.e.*, before they are one-year old. This seems to be a very unique phenomenon in the case of the insectivorous bats for no other author has recorded a similar feature in any of the microchiroptera. All authors are, however, agreed upon the fact that the bats have a very rapid period of growth even though they may remain sexually immature. But they believe that the bats come to sexual maturity during their second season.

Roland E. Miller (1939)[7] stated that "Young males of *Myotis lucifugus lucifugus and Myotis greisesens* do not enter into reproductive activity until their second spring". Harrison Matthews (1937)[5] working on the British Horse-shoe bats found that "The males do not reach their functional sexual activity until their second autumn".

And this view is confirmed by most of the authors working on the bats living in cold and temperate climates. This is probably because in these bats which inhabit the colder climates copulation takes place during autumn and the spermatozoa hibernate in the genital tract of the female during winter and fertilize the ova in the next spring. In the tropical bats on the other hand the problem of winter hibernation is bypassed and the males are nearly seven months old at the breeding season.

But Baker and Bird (1936)[1] working on the tropical bats in New-Hebrides stated that " None was definitely too small to be adult, but it is a well-known fact that bats become fully grown before they are sexually mature. The young could be detected by the size of their testes, which usually fell, fairly sharply into two groups as regards size. Those of the young naturally weighed about two milligrammes (both testes together), and sperms were never found in their epididymides ".

Van der Stricht (1910) was the only other author who recorded that in *Nyctalus noctula*, in Europe, the young females copulate in the same year as that in which they are born (from Baker and Bird).

The observations recorded in this paper give ample evidence to show that *Scotophilus wroughtoni* becomes sexually mature before it is one year of age. All the specimens collected in January and February were fecund, as indicated not only by the testis-weight but also by their microscopic structure. It would indeed be highly improbable that all collections during these months should not include a single immature specimen—the greater probability is that all males and females do become sexually active in their first year. Probably the variation in the period of copulation makes all the difference between the bats of the temperate and the tropical zones regarding the age at which they attain their sexual maturity.

As regards the determination of the age in these male bats it is impossible to decide from their sexual activity only. I have only to reiterate the statement of Harrison Matthews (1937) " The changes taking place in their genitalia do not throw any light on the duration of life of these animals, though presumably it is approximately the same in both sexes ".

A detailed study of the interstitial cells of the testis and the changes in the accessory reproductive organs in the male of this species will be dealt with in a separate paper.

Summary

1. Twenty-nine male specimens of the bat *Scotophilus wroughtoni* were collected during the different months of the year. Collection were made during two successive years from May 1945 to April 1947.

2. The weight of the testis of these bats increases from the month of December and reaches a maximum weight during February. There is an increase nearly four times in the testis-weight from September to February.

3. The diameter of the seminiferous tubules also increases corresponding to the increase in the testis-weight and progressively vigorous spermatogenetic activity is seen in the germ cells. The diameter of the tubules of the epididymis also increases during these months.

The occurrence of sperms in the tubules of the testis and the epididymis indicates that the breeding season is confined to these months.

4. The males, like the females, probably attain their sexual maturity in their first season, *i.e.*, before they are one year old.

ACKNOWLEDGEMENT

I am deeply indebted to Prof. M. A. Moghe for valuable guidance and help throughout my work. My thanks are also due to Mr. P. A. Ramakrishna Iyer, M.SC., Fellow of the National Institute of Science, Central College, Bangalore, for the technical guidance and training in the initial stages of my work.

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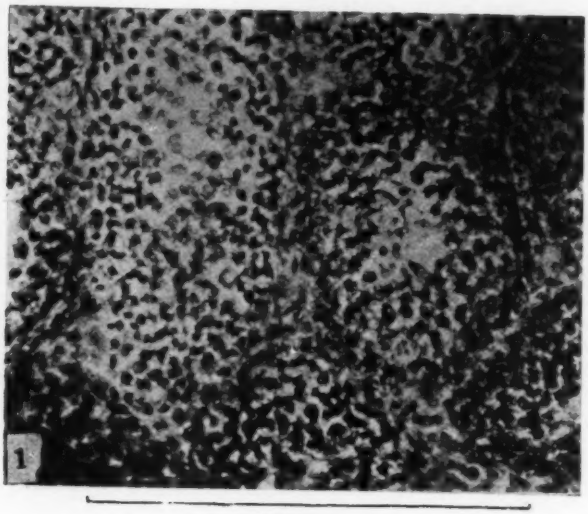

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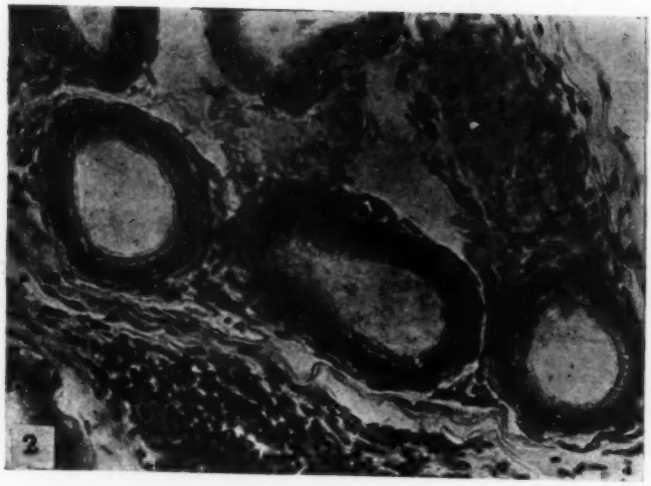

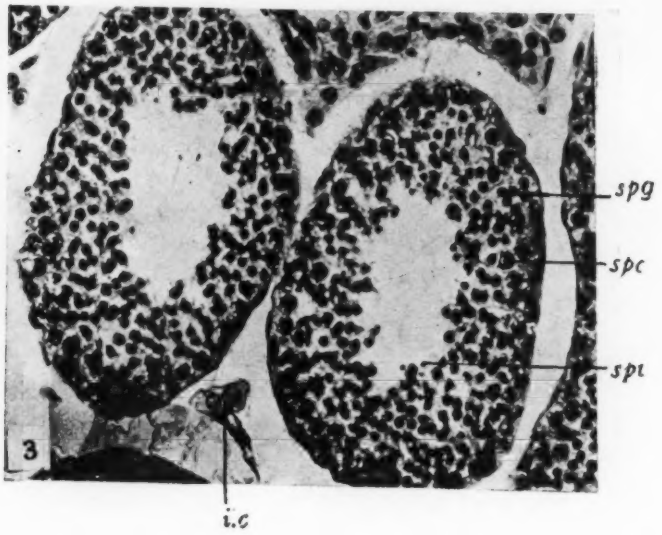
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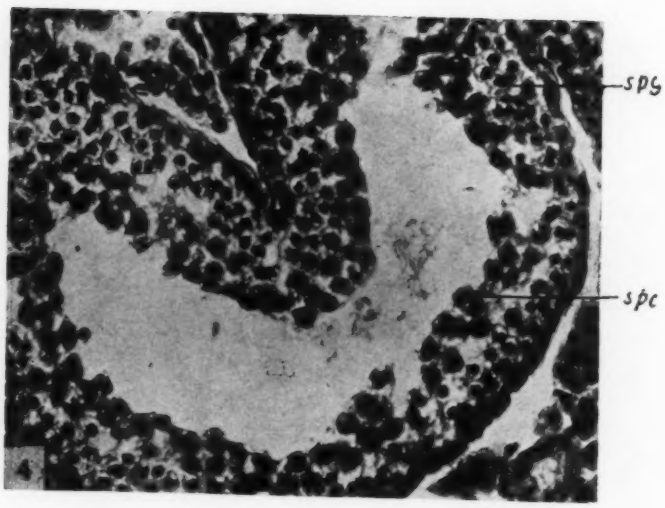
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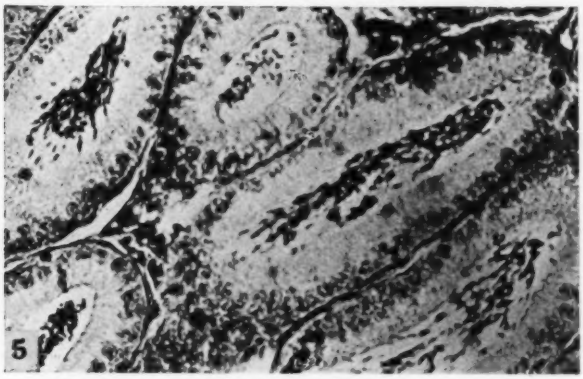
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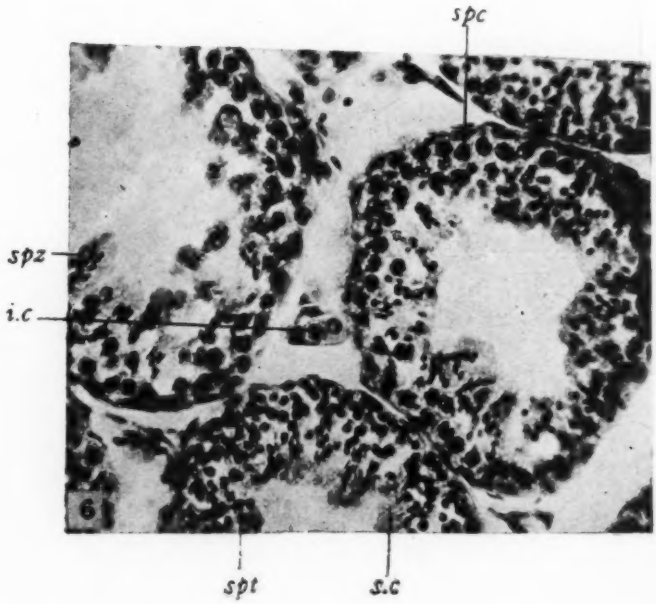
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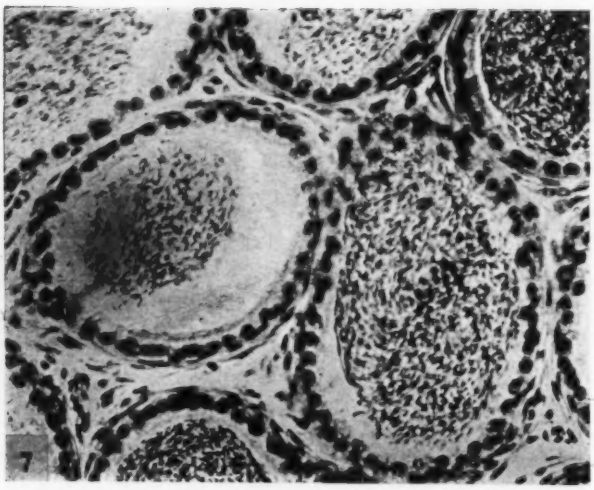

Explanation of Plates

All figures are photomicrographs. *Magnification of all figures is the same as of Fig.* 1.

Figs. 1–7. Fig. 1. Two seminiferous tubules from a transverse section of the testis of a specimen collected in May (17–5–1945). The tubules have no lumen and consist of resting spermatogonial cells. Fig. 2. Epididymal tubules of the same specimen. The tubules are empty, *i.e.*, contain no spermatozoa. There is a large amount of intertubular tissue. Fig. 3. Two seminiferous tubules from a transverse section of the testis of a specimen collected in December (7–12–1946). The cells are in various stages of spermatogenesis. The tnbules have a distinct lumen. Fig. 4. One tubule from a transverse section of the testis of a specimen collected in January (8–1–47). Note the large lumen. Most of the germinal cells are in the final stage of spermatogenesis. The spermatocytes are abundant. Fig. 5. Epididymal tubules of the same specimen showing the sperms in the lumen of the tnbules. The epithelium of the tubules is still columnar. The lumen is not so large as it is in a later specimen. Fig. 6. Seminiferous tubules from a transverse section of the testis collected in February (10–2–1946). Spermatids and the spermatozoa are abundant. Fig. 7. Epididymal tubules of the same specimen. The lumen is large and full of spermatozoa. The epithelial cells of the tubules are cubical instead of columnar.

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CTENOPLANA BENGALENSIS N. SP. FROM THE MADRAS PLANKTON

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(*From the University Zoological Research Laboratory, Madras*)

Received March 24, 1947
(Communicated by Prof. S. G. Manavala Ramanujam, F.A.SC.)

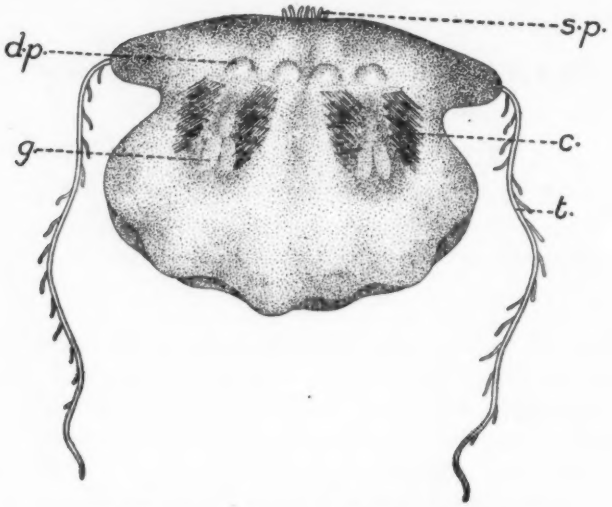
Introduction

From the time of the discovery of the first species, *Ctenoplana kowalevskii* by Korotneff in 1886, Willey (1897) described *Ctenoplana rosacea* and *Ctenoplana korotneffi*, Dawydoff* (1929 and 1936) recorded *Ctenoplana duboscqui* and *Ctenoplana perrieri* and Yoshi (1933) added *Ctenoplana maculomarginata* and *Ctenoplana muculosa*. Menon (1927) noted the occurrence of *Ctenoplana indica* in Madras, but as he did not publish any account of it, the *Ctenoplana* described in this paper is treated as a new species.

General Characters

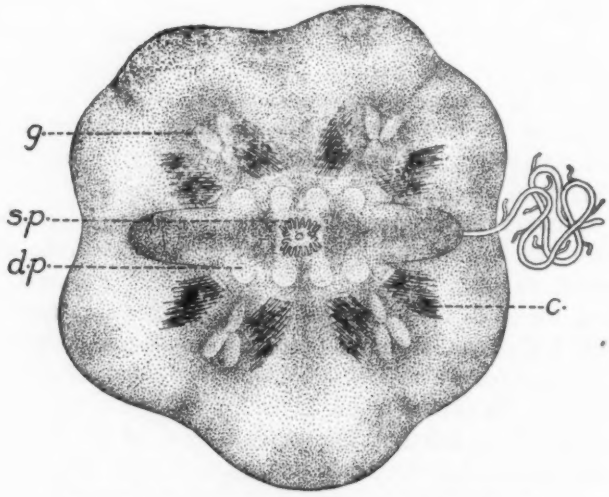
The specimen was found in the townet collection of the coastal plankton made off Madras on the 13th November 1946, and was studied alive in the laboratory for over a week. It endured rough handling while it was frequently pipetted from slide to dish and to the aquarium for purposes of observation and for changing the sea water. It was fed with live plankton and also with the flesh of prawn. The specimen measures 4 to 4·5 mm. and about 6 mm. when fully expanded. The tentacles when fully extended measure 15 mm. in length. While swimming, the lower or stomodæal part of the body appears distinct due to a constriction above it and then the oral edge is seen to be thrown into a number of lobes which come together and nearly close the mouth. In such a condition the body measures only 2 mm. in height and the form of the animal does not differ very much from that of the typical bell-shaped swimming ctenophore. While creeping, however, the mouth is opened out and the stomodæum is completely everted and extended all round the central region of the body which appears like a conical elevation much like a helmet. The outer margin of the flattened part is entire, devoid of the lobes seen when the animal is swimming. Nevertheless

* Dawydoff has also described *Planoctena agniæ*, *Planoctena yuri* and *Planoctena caulleryi*.



TEXT-FIG. 1. Side view of *Ctenoplana bengalensis*

the entire shape of the conical as well as the flat portions of the body keep changing owing to the constant extensions and contractions in different directions.



TEXT-FIG. 2. Dorsal view of *Ctenoplana bengalensis*

The creature moves about by swimming and creeping on floating objects. Swimming is entirely ciliary and the animal keeps all the comb

plates of the eight costæ in quick motion and moves with its aboral pole forwards trailing the tentacles fully extended. Creeping or gliding over objects like a flat worm is effected by movements of portions of the flattened region of the body. This is, however, facilitated by the working of the comb plates of a few of the ribs. But when the animal is about to leave the substratum the comb plates of all the costæ are brought into play and the central thick region is elevated to its maximum height until finally it rises and swims upwards. Occasionally the animal floats on the surface of the water in a fully expanded condition. The method of ingestion of food particles is interesting. When given a small piece of prawn flesh, the animal approaches it mouth forwards lying on the side of the body. It widens its mouth and turns over covering the bit with the pharynx and erecting the aboral part of the body. If the flesh is given when the animal is creeping, it glides over the food and engulfs it. During the process of ingestion the stomodæum becomes completely everted and flattened.

The coloration is neither marked nor uniform. It is of a yellowish brown colour with darker brown patches. These patches which are scattered along the margin of the flattened part and crowded in the region of the tentacle sheaths and the gonads appear to be of cells of the nature of melanophores and have numerous slender and anastomosing extensions by the contraction of which the shape and intensity of the patches are altered. The gonads are yellowish white and stand out conspicuously. There are also white pigment spots scattered all over the body but these are not very marked or defined owing to the dull background coloration. The tentacles and their branches are colourless and transparent. The comb plates are iridescent. Though observed in the dark room the animal did not show any capacity for phosphorescence in spite of repeated provocative handling. The animal is very sensitive to bright light and energetically contracts changing its form frequently.

Morphology

The eight costæ and their combs are very marked. Each rib is narrow and is flat at the aboral end while it tapers to a point orally. Each costa bears eight combs along its length. The width of the comb towards the oral end is narrower in accordance with the decreasing width of the rib, the last comb being extremely narrow bearing only a few cilia. The cilia are very long and are united up to about two-thirds of their length. The cilia of the upper combs are half as long as the costa itself. The cilia beat towards the mouth.

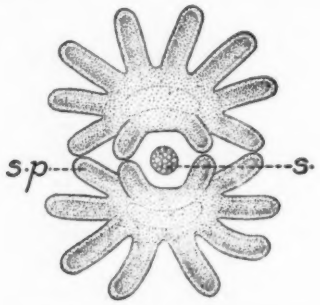
The statolith is small, spherical, opaque and granular in appearance. It is frequently hidden from view by the closing together of the finger-shaped

TABLE I

Species	Size	Colour	Shape	Sensory Papillæ	Other Features	Locality
C. kowalevskii Korotneff, 1886	7 mm. when moderately expanded. Height while swimming 3 mm.	Crimson with regular mahogany brown spots	Body in swimming attitude shaped like a truncated pyramid. Median dorsal surface concave. Free margin of skirt frilled	30-32 sensory papillæ (18–20 external and 12 internal)		West coast of Sumatra and South Annam (Bay of Nhatrang)
C. rosacea Willey, 1897	6 mm.	Crimson	Body in swimming attitude of a quadrilateral form. Median dorsal surface convex. Free margin of skirt plain	?		Eastern Archipelago of New Guinea
C. korotneffi Willey, 1897	6 mm.	Green	Body in swimming attitude roof-shaped. Median dorsal surface upraised into two upright end-knobs. Free margin of skirt slightly frilled	?		Eastern Archipelago of New Guinea
C. duboscqui Dawydoff, 1929	5·5 mm. when fully expanded. Height while swimming 2·5 mm.	Grayish white with the central thick region olive green or intense yellow olive sprinkled with yellow dots on the peripheral region. Tentacular sheath orange brown		23 sensory papillæ (15 external and 8 internal)	Costa with a single comb plate	South Annam (Bay of Nhatrang)
C. maculomarginata Yoshi, 1933	3 mm when moderately expanded and twice as large when fully extended	Pale yellowish green or light grayish olive with yellowish brown spots arranged at regular intervals along the margin		20–24 sensory papillæ		Misaki

C. muculosa Yoshi, 1933	5–8 mm. when moderately expanded and twice as large when fully spread out	Light greenish yellow or clear pink with 13 or 14 yellowish brown spots having dark purple pigment particles arranged along the margin		24 sensory papillæ		Misaki
C. perrieri Dawydoff, 1936	6 mm. Height while swimming 2·5 mm.	Bright emerald green with scattered orange vermilion spots arranged in clusters. Tentacular sheaths shining vermilion		24 sensory papillæ (14 external and 10 internal)		South Annam (Bay of Nhatrang)
C. indica Menon, 1927						Madras (Bay of Bengal)
C. bengalensis n.sp.	4–4·5 mm. when moderately expanded and 6 mm. when fully expanded. Height while swimming 2 mm.	Yellowish brown with dark brown patches along the margin of the flattened portion. Regions of tentacular sheaths and gonads dark brown	Free margin of skirt not frilled	20 sensory papillæ		Madras (Bay of Bengal)

sensory papillæ. There are twenty sensory papillæ. These papillæ are long and slender when extended and are capable of contraction and supple movements. The sensory region is elliptical with the semicircular ring of sensory papillæ bordering each side. The entire area containing the aboral sense organ, the polar fields and the sets of papillæ is frequently tucked in and thrust out especially when the animal is irritated. The papillæ and the polar fields are not pigmented.



TEXT-FIG. 3. The aboral sense organ

The two tentacles are long, highly contractile and bear uniseriate branches. These branches are regularly arranged, but decrease in length and number towards the distal extremity of the tentacle. The tentacle is extruded and withdrawn through the narrow mouth of the spacious tentacle sheath. Through the transparent sheath, when the pigmentation above it becomes lighter, the root of the tentacle can be seen at the proximal end of the sheath.

The gastrovascular system begins with the wide stomodæum or pharynx, the eversion of which is responsible for the planarian appearance of the creature. When not everted the stomodæum forms nearly half the height of the animal. When the animal is allowed to attach to a coverslip, an oral view into the interior of the œsophagus can be obtained. The wall of the œsophagus which is very short is produced inwards into a number of irregular highly contractile branching folds. Beyond the œsophagus, when it is widened, can be seen the stomach and the peripheral canals. Of these canals there are six on each side, those in the tentacular plane being wider. The canals branch repeatedly till they merge into the network of canals in the flattened part of the body. The stomach is spacious and is elongated along the tentacular axis. When the animal contracts its body eight sac-like projections, the dorsal papillæ, can be seen pushed out on the aboral side of the body. The anal pores and the anal canals could not be observed owing to the ramifying pigmentation.

The gonads are four in number situated in the four interradii between the tentacular and stomodæal axes.

General Remarks

The important taxonomic features of all the known species of *Ctenoplana* as can be gathered from a complete search of the available literature is given in Table I. A comparison of the characters of the Madras form with those of the other species of *Ctenoplana* shows clearly that the species described here is new to Science. In the number and arrangement of the sensory papillæ *Ctenoplana bengalensis* differs from all the known species. The sensory papillæ of *Ctenoplana kowalevskii, Ctenoplana duboscqui* and *Ctenoplana perrieri* are arranged in two sets, one internal and the other external. The arrangement of these papillæ in *Ctenoplana maculomarginata* and *Ctenoplana muculosa* appears to conform with that of *Ctenoplana bengalensis*, but there is variation in the number of the sensory papillæ. There is no information about the sensory papillæ of *Ctenoplana rosacea* and *Ctenoplana korotneffi*. Similarly the coloration of *Ctenoplana bengalensis* is markedly different from that of all the recorded species of *Ctenoplana*.

It is significant that this species also like all the known species of *Ctenoplana* has been collected from the plankton. *Ctenoplana kowalevskii* was found drifting in a current of water along with numerous *Porpita*. *Ctenoplana rosacea* and *Ctenoplana korotneffi* were taken from a cuttle bone which was floating and moving along the current. *Ctenoplana duboscqui* and *Ctenoplana perrieri* were collected from the plankton while *Ctenoplana maculomarginata* and *Ctenoplana muculosa* were obtained from floating sea weeds. From these recorded observations it is evident that *Ctenoplana* is essentially a pelagic form like the majority of the ctenophores, both during the swimming and creeping conditions, and inhabits the coastal waters. Mention may be made here that all the recorded species of *Planoctena* were also collected from the plankton except *Planoctena caulleryi* which was collected from an Octocorallian belonging to the genus *Xenia*.

Acknowledgements

Our thanks are due to Mr. C. Vedachalam for bringing the specimen to our notice and to Mr. S. Gopalan Nair for translating the French articles. We are also thankful to Dr. S. G. M. Ramanujam for lending us a few journals.

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* This has not been referred to in the original.

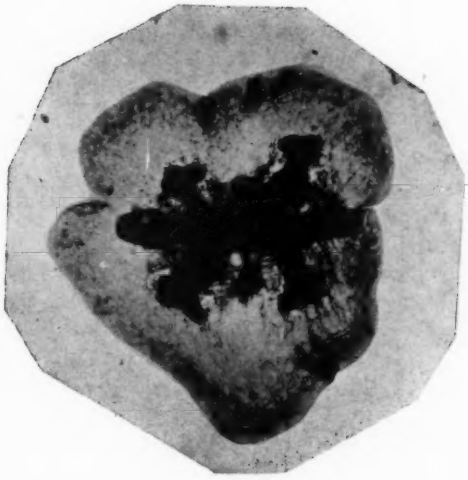
Explanation of Photographs

Photomicrograph 1. Dorsal view of *Ctenoplana bengalensis*.

„ 2. The aboral region showing the sense organ, sensory papillæ and dorsal papillæ.

Key to Lettering

c.	costa.	*s.*	statolith.
d.p.	dorsal papilla.	*s.p.*	sensory papilla.
g.	gonad.	*t.*	tentacle.



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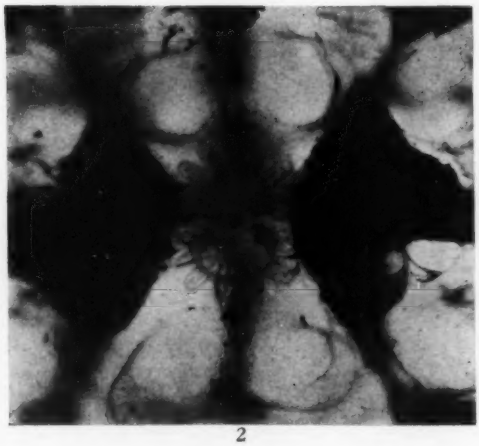


2

SOUTH INDIAN PHYCOMYCETES—I*

Pythium indicum Sp. nov. causing a Fruit Rot of *Hibiscus esculentus* Linn.

By M. S. Balakrishnan, M.Sc.

Received August 11, 1947
(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

Introductory

In November 1946, the writer received a few diseased fruits of *Hibiscus esculentus* L. collected by Mr. C. L. Sundararajan, B.Sc. (Ag.), from his estate at Podanur. It was stated that these specimens were representative of a type of decay responsible for considerable damage. Incidence of fruit rot was high during the months of October and November when there was very heavy rainfall with few sunny days and the reduction in yield amounted to over 50 per cent. The affected fruits showed large water-soaked, sunken, brownish lesions covered by a slight growth of fluffy white aerial mycelium. The tissues inside were found decayed and greatly softened with the result that the material gave a peculiar marshy odour. Microscopic examination revealed that the aerial mycelium consisted of non-septate hyphæ; moreover, the numerous oogonia with oospores in all stages of development and the accompanying antheridia found in portions of the aerial mycelium matted in severely rotted portions of fruits, indicated that the pathogen was in all likelihood a species of *Pythium*. This was confirmed when bits of aerial mycelium left overnight in sterile distilled water or soil leachate produced sporangia, their mode of formation and germination proving beyond doubt that it was a species of *Pythium* with filamentous sporangia. A survey of literature showed that no species of *Pythium* with filamentous sporangia has so far been recorded on this host. Further, this fungus also showed several interesting features and, therefore, a detailed study of the organism was undertaken.

The Causal Fungus

Detailed examination of diseased tissues showed that the pathogen was intracellular; the softened decaying tissue was occupied by branched

* Contribution from the I.C.A.R. School of Mycology, Agricultural Research Institute, Coimbatore.

non-septate hyphæ showing no definite orientation. At points where the hyphæ pierced the cross walls, they were markedly constricted. Pure cultures of the fungus were easily obtained by placing surface-sterilized bits of diseased tissue on oatmeal agar plates and transferring portions of the mycelium from the margin of the resultant growth to sterile media in tubes. Raper's technique was adopted to free the cultures from bacteria.

The fungus grew well on most of the agar media, growth being particularly luxuriant on oatmeal, French-bean and carrot agars. Of these three, oatmeal agar was found best and subsequent pure cultures were maintained on this medium. Growth was very rapid on most of the media at laboratory temperature (26–27° C.) a 10 cm. Petri dish being completely covered with dense aerial mycelium in 36 hours. Aerial mycelium was less luxuriant on synthetic media such as Raulin's agar or plain water agar. At laboratory temperature this isolate grew faster than fresh isolates of *P. aphanidermatum* (Eds.) Fitz. and *P. graminicolum* Subr. indicating that this must be one of the fastest growing tropical species of the genus known.

Morphology.—The hyphæ varied from 4 to 12 μ in diameter, mostly 8 to 10 μ, and were profusely branched. The axial hyphæ were very stout, often measuring over 10 μ in diameter, while the lateral branches were comparatively slender, measuring 4 to 6 μ in diameter. Clavate and falcate appressoria, either simple or compound, were formed in abundance wherever the hyphæ came into contact with obstruction, *i.e.*, the side or bottoms of a Petri dish or the side of a test-tube. These appressoria were considerably broader than the parent hyphæ often exceeding 20 μ in thickness, and were filled with dense granular protoplasm (Fig. 1, *A*, *B*). The hyphæ were for the most part non-septate, becoming septate only in the older portions of the intra-matrical mycelium and were filled with homogeneous finely granular protoplasm without any inclusions or vacuoles.

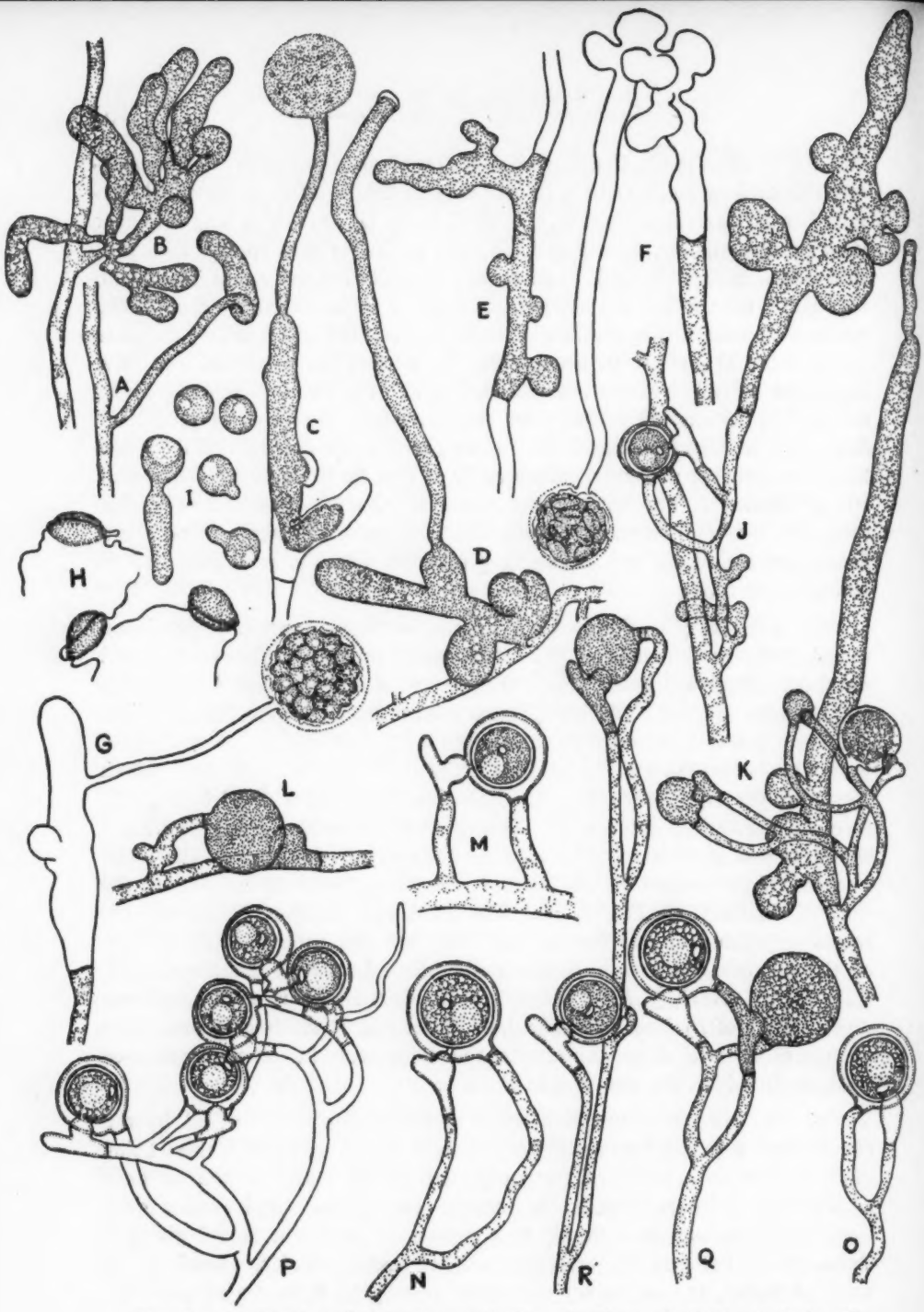
Asexual reproduction was easily induced by excising small bits from an oatmeal agar-plate culture of moderate thickness and transferring them to a shallow layer of sterile distilled water or steamed soil leachate in a sterile Petri dish. In such preparations, abundant sporangia were produced in 12 to 15 hours. Experience showed that the production of sporangia was more rapid in soil leachate than in distilled water; also, more sporangia germinated indirectly (by producing zoospores) in soil leachate than in distilled water.

The sporangia were inflated structures, rarely lobed and digitate but never forming such intricate complexes as in *P. aphanidermatum*. They were always thicker than the parent hypha, ranging from 15 to 20 μ in diameter

and 100 to 250 μ in length and were either terminal or intercalary. The sporangia opened by an evacuation tube of varying length, either subterminal, terminal or lateral in position (Fig. 1, *C*, *D*, *E*, *F* and *G*), usually as long or longer than the sporangium, occasionally shorter, but never very short. Delimitation of the zoospores took place within a vesicle formed at the tip of the evacuation tube which was slightly wider at the tip of dehisence than at the point of its origin. The number of zoospores formed within a single vesicle varied from 25 to 150 depending on the size of the sporangium. The zoospores actively swarmed within the vesicle for about a minute, being released by its rupture. They were reniform, with a prominent groove or hilum and possessed two equal cilia inserted on the groove (Fig. 1, *H*). When free swimming the zoospores measured 12 to 15 μ by 10 μ and when encysted 10 μ in diameter. They always germinated by the production of 1 to 3 germ tubes (Fig. 1, *I*). No case of repeated emergence was seen. Under unfavourable conditions the sporangia germinated directly, producing one to several germ tubes.

On oatmeal and French-bean agars the fungus promptly developed sexual bodies in abundance. The first formed oogonia were always terminal on short lateral branches. These oogonia were accompanied by typically monoclinous antheridia which originated a short distance below the oogonium and were borne terminally on a straight stalk. In shape, the antheridia were filamentous-clavate, only slightly thicker than the stalk from which they were cut off. Very often, the antheridia possessed a distal lobe giving them a characteristic appearance. Though most of the first formed antheridia were terminal in position, they were also sometimes intercalary, the distal segment of the antheridial stalk usually remaining short at least up to the time of fertilization (Fig. 2, *G*). The most characteristic feature of the sexual apparatus in this fungus, however, was the pronounced curvature of the oogonial stalk towards the antheridium borne on a straight stalk (Fig. 1, *J*, *K*, *N*, *O*, *Q*, *R*). This feature is shared by this species with two other species of *Pythium*—*P. indigoferæ* Butler and *P. deliense* Meurs. This phenomenon is in sharp contrast to the behaviour in other species of *Pythium* in which the converse is the rule.

As the cultures grew older, more sexual bodies were formed by the lateral branch which produced the primary sexual apparatus, these being often formed as a result of further growth of the stalks of the primary antheridium and oogonium, so that often 5–10 oogonia and their antheridia were borne together in a closely knit cluster on the fertile lateral giving a characteristic habit to the mycelium. When such secondary sexual bodies were produced, the secondary oogonium usually took its origin from the

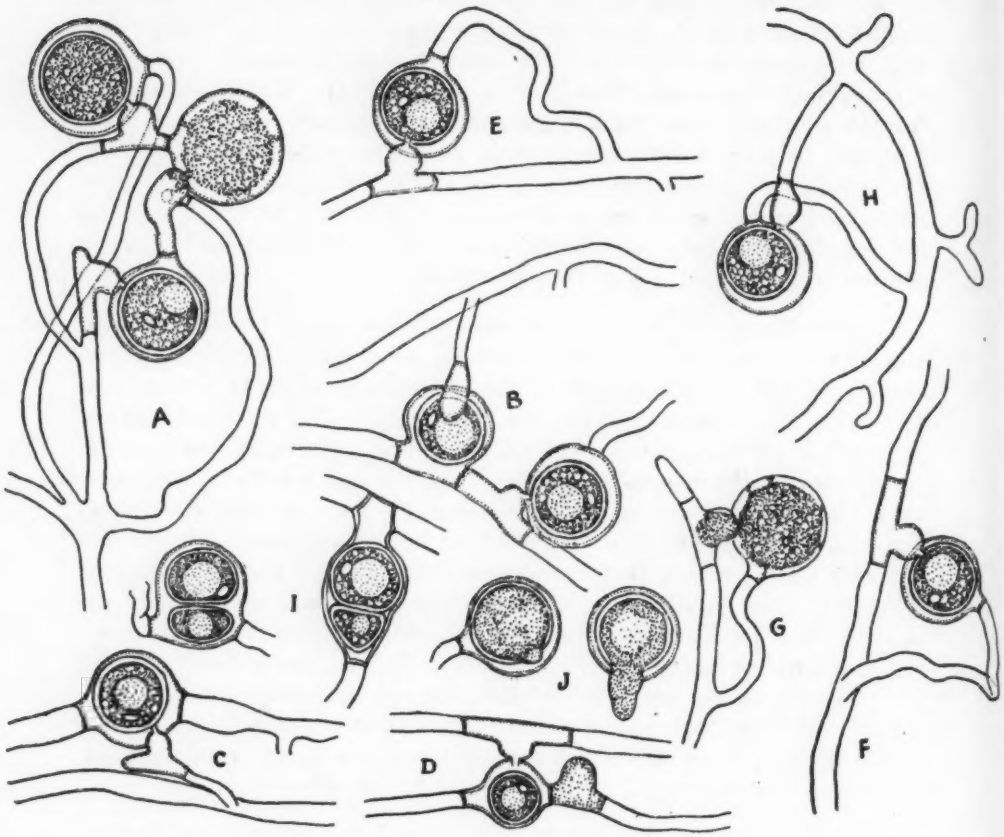


TEXT-FIG. 1. Sexual and asexual reproductive structures of *Pythium indicum*. The magnification is × 480 in the case of figs. *A–G*, *J*, *K*, *P* and *R* and × 680 in the case of figs. *R*, *I*, *L*, *M*, *N*, *O* and *Q*.

primary antheridial stalk and was fertilized by an intercalary antheridium formed on the stalk of the primary oogonium adjacent to it. This process was repeated till finally a cluster of sexual bodies was formed round the primary sexual apparatus (Fig. 1, *P*, *Q*, Fig. 2, *A*). Exceptions to this general rule were also found; sometimes the primary antheridial stalk branched to form another antheridium while the primary oogonial stalk produced an oogonium and so on (Fig. 1, *R*). In all cases there finally results a cluster of sexual organs borne on a single fertile lateral. As far as the writer is aware, such a development of sexual bodies giving rise to clusters has not been observed in any other species of *Pythium*.

Again, though the first formed oogonia were always terminal accompanied by strictly monoclinous antheridia, considerable diversity in the shape, position and relationship of the antheridium to the oogonium was seen in the later formed sexual bodies. As already stated the typical sexual apparatus in this form consisted of a terminal oogonium which was strongly curved towards the monoclinous antheridium borne terminally on a straight stalk. Sometimes, however, this relationship was not seen, the antheridium and oogonium being borne on parallel branches without any curving of the oogonial stalk towards the antheridium (Fig. 1, *M*). Another variation commonly seen was that the oogonium was intercalary, sub-spherical or barrel-shaped, fertilized by a diclinous antheridium which in its turn may be either terminal or intercalary (Fig. 2, *B*, *C*, *D*). Or, the antheridium may be intercalary on the parent hypha itself, the oogonium being brought into contact with it by a pronounced curvature of the oogonial stalk (Fig. 2, *E*, *F*). Such a diversity of antheridial relationship is not seen in the two congeneric forms mentioned above—*P. indigoferæ* and *P. deliense*.

The oogonia varied in diameter from 12 to 24 μ (av. 19·6 μ) and were usually accompanied by only one antheridium. After fertilization an oospore was formed inside the oogonium showing the internal organization characteristic of the ripe oospores of most species of *Pythium*, a single reserve globule surrounded by densely granular protoplasm in which was imbedded a spherical or kidney-shaped refringent body. Developmental irregularity sometimes led to the formation of two oospores within a single oogonium (Fig. 2, *I*). The oospores were aplerotic with a moderately thick wall and varied in diameter from 10 to 20 μ (av. 15·94 μ) and germinated by the production of a germ tube (Fig. 2, *J*). Though the sexual bodies were borne separately from the sporangia, they were often found associated with sporangia as reported by Butler for his *P. indigoferæ*, a congeneric form. (Fig. 1, *J*, *K*).



TEXT-FIG. 2. Sexual reproductive structures of *P. indicum*. × 680.

Cultural.—The growth of the fungus was studied on the following culture media used as agar slants in test-tubes in comparison with a type culture of *P. indigoferæ* obtained from the National Collection of Type Cultures, Delhi, and a fresh isolate of *P. aphanidermatum* isolated by the writer from rotting tomato fruits collected at Coimbatore. There were three replications for each medium used and growth was compared after 15 and 22 days at 20° C. The results of these studies are given in the table below:

It will be seen from the above table that the *Pythium* isolated from *H. esculentus* is the most vigorous grower of the four congeneric species compared here. *P. aphanidermatum* also shows abundant aerial mycelium

TABLE I

Comparison of growth of *P. indigoferæ*, *P. aphanidermatum*, *P. deliense** and the *Pythium* isolated from *Hibiscus esculentus*

Culture medium used	*P. aphanidermatum*	*P. indigoferæ*	*P. deliense*	*P. sp.* (*Hebiscus esculentus*)
1. Oatmeal agar ..	Abundant cottony aerial mycelium filling the entire tube flush with the top of agar slant	Meagre aerial mycelium forming a mat on the surface of agar-growth mosity submerged	Moderate aerial mycelium, thin in upper half, dense in lower half though not filling it	Abundant cottony aerial mycelium filling whole tube; sharp horizontal demarcation flush with top of agar slant appearing as a solid plug
2. French bean agar	do	do	..	do
3. Carrot agar ..	Abundant aerial mycelium though not so luxuriant as in oats agar; filling tube with nearly horizontal demarcation	Growth poorer than in oatmeal or frenchbean agars. Meagre aerial mycelium, growth submerged	Moderate aerial mycelium, thin in upper half, more dense in lower half	do
4. Nutrient agar ..	Moderate aerial mycelium filling half of tube, forming a dense mat on the surface of the slant	Very poor growth, entirely submerged, no aerial mycelium	..	Moderate aerial mycelium, more abundant than *P. aphanidermatum* in this medium forming a thick mat on the surface of the agar slant
5. Raulin's agar ..	Growth poorer than in nutrient agar, moderate aerial mycelium matted on the surface	Practically no groath even after one month	Extremely meagre aerial mycelium. More dense in upper than in lower half	do
6. Water agar ..	do	Very poor growth; no aerial mycelium	..	do

* *Note.*—The data given for *P. deliense* are taken from Meurs' account of the fungus.

TABLE II

Inoculation Experiments with the Pythium *isolated from* Hibiscus esculentus

Plant used for the experimental	Parts inoculated	Result	Remarks
1. *Nicotianæ tabacum* L.	Terminal bud, stem and leaves (unwounded	–	
2. do ..	do (wounded)	+	Large brownish lesions were seen on the second day after inoculation. These spread rapidly. Aerial mycelium was seen on the affected parts on the 4th day after inoculation
3. *N. glutinosa* L.	Terminal bud, stem and leaves (unwounded)	–	
4. do ..	do (wounded)	+	Symptoms same as in *N. tabacum*
5. *Solanum melongena* L. ..	Stem, terminal bud and young leaves	+	Dieback was seen on the fourth day after inoculation. Only young portions of stem and young leaves were infected
6. do ..	Fruits (unwounded)	–	
7. do ..	do (wounded)	+	Sunken water-soaked lesions appeared round the point of inoculation on 2nd day and spread rapidly. The whole fruit was rotted in 7 days. Profuse aerial mycelium was seen on the fruit after the 4th day
8. *S. nigrum* L. ..	Terminal bud	+	Severe dieback was seen on the 3rd day after inoculation. The plant was killed in 10 days
9. *Capsicum annuum* L.	do	+	do
10. *Licopersicum esculentum* Mill	do	+	Dieback not so severe as in *S. nigrum* and *C. annum*. The plant was killed in 15–18 days
11. do ..	Fruits (unwounded)	+	Brownish lesions seen round the point of inoculation on 2nd day; fruits completely rotted in 5–7 days
12. do ..	do (wounded)	+	Infection more rapid than in unwounded fruits. Fruits were completely rotted in 4 days
13 *Datura fattuosa* L.	Terminal bud	–	
14. *Petunia* sp. ..	do	+	Severe dieback was seen. The plants were killed in 7–10 days
15. *Carica papaya* L.	Fruits (unwounded)	+	Fruits were completely rotted in a week
16. do ..	do (wounded)	+	Infection more rapid. Fruits were completely rotted in 5 days
17. *Cucurbita maxima* Duchesne ..	do (unwounded)	+	Infection more severe than in all other fruits tried. Even large fruits were completely rotted within a week

TABLE II—*Contd.*

Plant used for the experiments	Parts inoculated	Result	Remarks
18. *Cucurbita maxima* Duchesne ..	Fruits (wounded)	+	Fruits were completely rotted in 4 days. Abundant aerial mycelium similar to that described by Drechsler (1925) for cottony leak of cucumbers
19. *Zea mays* L. ..	Base of stem (wounded)	+	Severe stalk rot was evident three days after inoculation. The plants fell over and died in a week
20. *Pennisetum typhoides* Rich.	do	–	
21. *Amaranthus gangeticus* L.	Terminal bud	+	Infection severe. Dieback resulted and the plants were killedin 4–5 days
22 *Vigna catjang* Endl.	do	+	Infection slight confined to yonuger portions alone
23. *Hibiscus esculentus* L.	do (Seedlings in 2-leaved stages)	+	Both cotyledonary leaves drooped six hours after inoculation. Rotting was evident in 12 hours and rot spread rapidly dowuwards, killing the plant in 72 hours. Profuse aerial mycelium was seen on rotted parts.
24. do ..	do (seedlings in 4- and 6-leaved stages)	+	Infection evident 15 hours after inoculation. Dieback very severe, plants being killed within 5 days
25. do ..	Axils of leaves of young plants (8- leaved)	+	The petioles and laminæ took infection and rotted in 36 hours. A lesion about 1″ in diameter was seen at the mode. No further spread of infection up or down the stem was seen
26. do ..	Fruits (wounded)	+	Infection very rapid, fruits being completely rotted in 3 days
27. do ..	do (uhwounded)	+	do

though to a slightly lesser degree. According to Meurs (1934) the aerial mycelium of *P. deliense* is less luxuriant than that of *P. aphanidermatum*. *P. indigoferæ* is the poorest grower, presenting a marked difference in cultural behaviour from the other three species.

Pathogenicity: Numerous inoculation experiments were carried out to test the pathogenicity of this species and aiso to get an idea of the host range. These experiments were carried out on potted plants one to two months old except in cases where it is stated otherwise. The plants were well washed with sterile distilled water before inoculation and kept covered with bell jars after inoculation so as to maintain a high level of humidity. Fruits to be inoculated were thoroughly washed in an aqueous solution of mercuric

chloride (1 in 1,000) and several changes of sterile distilled water and kept in sterile moist chambers. In all cases plants and fruits similarly treated and kept under the same conditions without the inoculum were kept as controls. These ramained healthy and unaffected throughout.

The results indicate that this species is likely to have a very wide host range. Only one species of Pythium—*P. de Baryanum* Hesse—has been recorded on *H. esculentus* so far (Ramos, 1926).

Taxonomy.—As stated already, the present species shows a close relationship to *P. indigoferæ* and *P. deliense* in the bending of the oogonial stalk and appended oogonium towards the antheridium. The antheridial stalk always remains straight. This phenomenon is in sharp contrast to the behaviour in other species of *Pythium* where the converse is the rule. However, it also exhibits certain differences from both the abovementioned species. From *P. indigoferæ* it can be easily differentiated by its more vigorous and robust growth on most agar media and especially on synthetic media like Raulin's agar in which *P. indigoferæ* is unable to grow at all. Moreover, in *P. indigoferæ* the oogonia are always terminal whereas in the form under discussion they are frequently intercalary; also, intercalary antheridia as met with in the present form are not seen in *P. indigoferæ*. Further, though much stress cannot be laid on the fact that the *Pythium* on *H. esculentus* produces abundant sporangia as against the rare occurrence of these structures in *P. indigoferæ*, it is useful as an additional distinguishing feature between the two species as also the fact that in this species the evacuation tube is as long or longer than the sporangium, never very short as described by Butler for *P. indigoferæ*.

From *P. deliense* which comes nearest to it the *Pythium* on *H. esculentus* differs in the production of abundant aerial mycelium on most culture media as opposed to the 'moderate' aerial mycelium of *P. deliense*. This difference is most marked in synthetic media as Raulin's agar. Meurs (*loc. cit.*), who first described *P. deliense* from tobacco in Sumatra says that it did not grow well in Raulin's agar and produced meagre aerial mycelium. The present form, however, produces fairly luxuriant aerial mycelium on Raulin's agar though not quite as abundantly as in richer media. Further, in this species, the sexual bodies and sporangia occur together, a feature which is absent in *P. deliense*, where sporangia and sexual organs occur separately. This feature is so characteristic of *P. deliense* that both Meurs and Middleton (1943) state it to be diagnostic for that species. The production of appressoria in abundance is another point of difference between these two species. Meurs says that appressoria are conspicuously absent in *P. deliense*. Finally,

the evacuation tube in the *Pythium* on *H. esculentus* is never shorter than the sporangium; in *P. deliense* on the other hand, it is often very short.

This fungus is also closely allied to *P. aphanidermatum*. The oogonia are similar in size and position and in instances when the oogonial stalk is not bent towards the antheridium as in cases of both sexual organs arising on parallel branches of the same parent hypha, or when the antheridium is diclinous, some difficulty may be experienced in distinguishing the two species, especially if the antheridium is intercalary as sometimes happens in this species. However, though intercalary antheridia are common to both species, they are more frequent in *P. aphanidermatum* and are typical of that species. Further, the antheridia in *P. aphanidermatum* are typically diclinous and intercalary and only occasionally monoclinous and terminal. As stated previously, the first formed antheridia in the present form, however, are typically terminal and monoclinous and are a characteristic feature of the fungus; intercalary and diclinous antheridia are atypical and infrequent. This feature can be taken as a very stable and satisfactory criterion for the differentiation of these two species. Finally, the *Pythium* on *H. esculentus* is further distinguished from *P. aphanidermatum* by the characteristic clustering of the sexual organs on the fertile lateral, the smaller size of the oogonia and oospores,—(oogonia 27 μ and oospores 26·55 μ in *P. aphanidermatum* as against 19·6 μ to 15·94 μ in this species), the less complex nature of the sporangia and the typical curvature of the oogonial stalk towards the antheridium which is always borne on a straight stalk.

The differences mentioned above are, in the writer's opinion, sufficient to consider this fungus distinct from *P. indigoferæ*, *P. deliense* and *P. aphanidermatum*. It is, therefore, presented as a new species, *Pythium indicum*.

Pythium indicum sp. nov.: Hyphæ non-septate when young, irregularly septate when old, measuring 4–12 μ, mostly 8–10 μ, in diameter, sporangia terminal, infrequently intercalary, inflated filamentous, up to 250 μ long, wider than the parent hypha, provided with lateral lobes though never forming intricate complexes. Zoospores 25 to 150, reniform, laterally biciliate, measuring 12–15 μ by 10 μ while free swimming and 10–12 μ in diameter when encysted, monoplanetic, germinating by the production of 1–3 germ tubes. Zoospores delimited within a vesicle formed at the tip of an evacuation tube of varying length, as long or longer than the sporangium, never very short, with an enlarged tip of dehiscence; evacuation tube usually terminal though at times sub-terminal or intercalary.

Oogonia spherical, sub-spherical or barrel-shaped, terminal or intercalary, smooth walled, measuring 12 to 24 μ (av. 19·6 μ) in diameter.

Antheridia typically monoclinous, rarely diclinous, single, the antheridial stalk usually straight with the oogonial stalk bent towards it. Rarely the antheridial and oogonial stalks may be parallel branches of the same parent hypha. Antheridial cell filamentous-clavate, terminal, rarely intercalary, often with a distal lobe, making apical contact with the oogonial wall. Fertilization tube moderately thick and clearly visible. Oospores smooth, aplerotic, with a moderately thick wall containing a single reserve globule and a single refringent body. Oospores measuring 10·20 μ (av. 15·94 μ) in diameter, germinating by a germ tube.

Causing fruit rot of *Hibiscus escutentus* L. Podanur, Coimbatore, South India, collected by C. L. Sundararajan.

Type culture deposited in the Government Mycologist's collection of stock cultures, Agricultural Research Institute, Lawley Road P.O., Coimbatore, South India.

Pythium indicum sp. nov.—Hyphæ juvenes non-septate, veteres irregulariter septatæ, 4–12 μ, plurimum 8–10 μ diam.; sporangia terminalis, rare interposita, inflatis-filamentosa, ramosissima, ca. 250 μ longa, latora quam hypha parens, tubulis vel zoosporis germinantia; zoosporia reniformis, biciliatis, 12–15 × 10 μ diam. natantia, 10–12 μ cystidiosacta, monoplaneticis, germinantibus uno vel tribus tubulis; oogonia terminalia, rare interposita, globosa vel sub-globosa, 12–24 μ (av. 19·6 μ) diam.; antheridia monoclina, rare diclina, solitaria, pedicilii recti, pedicellus oogoniæ curvatus ad antheridium, interdum pedicilli antheridium oogoniæ parallelli; oosporæ aplerotícæ, cuticulis mediocriter crassa, unicam globulum et corpus nicans habentes, 10–20 μ (av. 15·94 μ) diam, tubulis germinantes.

Causat putredinem fructus *Hibisci esculentis*, Podanur, Coimbatore, South India. Leg. C. L. Sundararajan.

ACKNOWLEDGEMENTS

The writer wishes to express his indebtedness to Mr. K. M. Thomas, B.A., M.Sc., D.I.C., Government Mycologist, for his kind criticism and guidance throughout the investigation. His thanks are also due to the Indian Council of Agricultural Research for the grant of a research fellowship during the tenure of which this investigation was carried out, to Revd. Fr. M. Singarayar of St. Joseph's Seminary, Coimbatore, for the latin translations, and to Dr. T. S. Sadasivan, M.Sc., PH.D., Director, University Botany Laboratory, Madras, for kindly going through the manuscript and offering suggestions.

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INOCULATION TRIALS WITH *PYTHIUM INDICUM* SP. NOV.

FIG. 1. Inoculated (A) and healthy (B) seedlings of *Hibiscus esculentus*.

Note.—In (A) the two seedlings in the foreground show die-back due to inoculation on the terminal bud ; in the case of the two seedlings in the background inoculation was in the axils of the two lowest leaves which alone have rotted.

FIG. 2. Healthy (A) and inoculated (B) plants of *Petunia* sp. Note the dieback ; both plants were of equal height when inoculated.

FIG. 3. Fruits of *Cucurbita maxima* Duch. (A), wounded inoculated, (B), control and (C), unwounded inoculated. Photograph taken 5 days after inoculation.

FIG. 4. Healthy (A) and inoculated (B) plants of *Zea mays* L. showing stalk rot and falling over of the infected plant.

SUMMARY

A species of *Pythium* with filamentous sporangia was isolated from rotting fruits of *Hibiscus esculentus* L. collected at Podanur, Coimbatore District, South India. A detailed study of the fungus showed it to be a form related to *Pythium indigoferæ*, *P. deliense* and *P. aphanidermatum* but possessing certain characteristics which mark it as different from these three species. On account of these differences, it is considered a new species and named *P. indicum*. Inoculation experiments showed it to have a wide host range. Only one other species of Pythium—*P. de Baryanum* Hesse—has been so far recorded on *Hibiscus esculentus*.

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STUDIES ON THE MORPHOLOGY, PHYSIOLOGY AND PARASITISM OF THE GENUS *PIRICULARIA* IN MADRAS*

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Received November 10, 1947

[Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., (Lond.) F.A.Sc.]

The genus *Piricularia* belonging to the group *Hyphomycetes* is well known as the causal organism of the 'blast' disease of rice (*Oryza sativa* Linn.), 'ragi' (*Eleusine coracana* Gaertn.), and 'tenai' or the Italian millet (*Setaria italica* Beauv.) bringing about a heavy loss of food grains—as heavy as 76% or more (McRae, 1922). Other important hosts are the banana ('pitting disease'—Hoette, 1936; Van Hook, 1926), *Triticum vulgare* Vill. (Anstead, 1924), *Theobroma cacao* Linn. (Saccardo, 1875) and *Zingiber officinale* Rosc. (Nisikado, 1927). Several grasses also serve as hosts.

The genus has a wide distribution, being present in all tropical regions, especially the rice-growing tracts. Work on *Piricularia* has been carried out in all these regions, chiefly Italy, Japan and India and rice being the most important of the hosts, *Piricularia* on rice (called *Piricularia oryzæ* Cav.) has received most attention. This form which causes the 'brusone' of rice (as the disease is called in Italy) was first recorded in Italy by Briosi e Cavara (1892) and much work has since been done on this fungus. In Japan work on 'blast' of rice (called 'Ine Imochibyo') was started by Hori (1898) and was continued by Kawakami (1901, 1902) and Miyake (1909, 1910). Much useful work on the host parasite relationship, leaf anatomy in relation to resistance, entry of the pathogen into the host, viability, breeding for resistance and methods of control was being done in research centres like Hokkaido and Formosa by numerous workers. Nisikado (1917, 1927) has made an attempt to assign a few Japanese isolates of *Piricularia* from different hosts to their systematic position within the genus with the help of their morphology, physiology and parasitism. In India the Madras Department of Agriculture has been tackling the 'blast' disease of cereals since 1918, when it was first recorded (McRae, 1920) as a serious outbreak in the Tanjore delta; much work has been done on the economic side of the problem, *viz.*, devising methods of control, breeding for resistance, varietal trials for selecting resistant varieties of cereals and cultural practices to eliminate the disease (McRae, 1920–1923; Sundararaman, 1922–1936; and Thomas, 1930, 1931 and 1936–1941).

* Formed part of a thesis accepted for the M.Sc. degree of the University of Madras.

The present communication includes a study of the morphology, physiology and parasitism of four isolates of *Piricularia* isolated from important cereals like *Oryza sativa* Linn., *Setaria italica* Beauv., and *Eleusine coracana* Gaertn., and the grass *Digitaria marginata* Link.

Morphological Studies

Materials and Methods.—*Piricularia* was isolated from infected leaf tissues of rice (*Oryza sativa* Linn.—strain Adt. 10), 'ragi' (*Eleusine coracana* Gaertn.—strain E.C. 593 of the Millets Specialist, Coimbatore), 'tenai' or the Italian millet (*Setaria italica* Beauv.—local Coimbatore variety) and *Digitaria marginata* Link by single spore isolation method. Stock cultures were maintained on oat-meal-agar slants and on sterilised leaf bits of the respective hosts in Roux tubes. All work, which necessitated the study of fresh mycelia or spores direct from the hosts, was done during November to January, when the crop at Coimbatore showed maximum infection in the fields.

Symptoms of infection.—In rice, 'ragi', 'tenai' and *Digitaria* the fungus causes the characteristic leaf spots. In rice and 'ragi', in addition to the leaf spots in their younger stages, there is a darkening of nodes and necks of earheads after the flowering of the crops. The blackening of the necks and nodes is, however, absent in the case of 'tenai' and *Digitaria*. That the attack by *Piricularia* is restricted to the foliage in *Digitaria* sp. has been observed by Hansford (1943) in Uganda.

Nature of leaf-spots.—Spindle shaped dark-brown leaf spots are met with on leaves of rice, 'ragi' and *Digitaria* while spots on the leaves of 'tenai' are more or less circular. The spots invariably appear on either side of the mid-rib. On leaves of rice and 'ragi' the spots are found to be 1 to 3 cm. long. Weather conditions favouring development, neighbouring spots coalesce and very long spindles are formed and the leaf tissue rots in the middle and gets torn. The spots are greyish in the centre and brownish in the periphery. In 'tenai' the leaf spots are smaller and scattered. The spots are about 2 to 5 mm. in diameter, light brown in the centre and dark brown in the periphery.

Mycelia.—There is no appreciable difference between the mycelia of the different isolates. They are all thin, hyaline and straight when young. In older cultures they are thicker and attain a slightly brownish tinge and are variously contorted, developing swellings. In all the isolates the breadth of the mycelium varies from 1·4 to 5·8 μ.

Conidiospores.—The spores of all the isolates are hyaline and top-shaped. They are mostly three-celled (rarely two- or four-celled). The

spores may be straight or slightly bent. Some of them are very long and narrow while some are fairly broad. In any case the spores are longer than broad. They are borne in a scorpioid manner on conidiophores which are hyaline, their septa being very prominent. The fungus puts forth the conidiophores through the stomata of the affected parts of the plant and conidia are exposed to the air. In leaves the conidia are met with in the spotted region both on the upper side and on the lower side of the leaf-blade but more abundantly on the upper side. The spores vary in length from about 19 to 37 μ and in breadth from 7 to 15 μ and show no appreciable difference between the isolates.

The following table gives the average measurement of 200 spores taken of the four isolates:

TABLE I

(Measurements expressed in microns)

Pircularia from		Spores from the living host		From sterile leaf bits	
		Length	Breadth	Length	Breadth
Rice ..	Mode	26·56	12·45	24·90	11·62
	Mean	29·22	12·63	26·35	11·58
	Range	20·75 to 37·35	10·79 to 14·94	21·58 to 32·37	9·13 to 14·11
'Tenai' ..	Mode	24·90	12·45	24·90	11·62
	Mean	24·00	12·00	23·70	11·37
	Range	19·92 to 31·54	9·96 to 13·28	19·92 to 30·50	9·13 to 14·11
'Ragi' ..	Mode	24·50	11·40	24·20	11·40
	Mean	23·60	11·20	23·40	11·30
	Range	19·50 to 30·00	9·50 to 14·50	19·30 to 30·20	9·50 to 14·50
Digitaria	Mode	24·00	11·20	24·20	11·30
	Mean	23·80	11·00	23·60	11·10
	Range	19·20 to 30·30	9·40 to 14·50	19·30 to 30·00	9·50 to 14·40

In all the four strains the end cells of the spores germinate readily when kept in a drop of water in a Van Tieghem cell.

Chlamydospores.—These are almost alike in all the isolates, round in shape, thick-walled, varying from 4 to 10 μ in diameter. These are brownish green in colour. Both terminal and intercalary chlamydospores are met with.

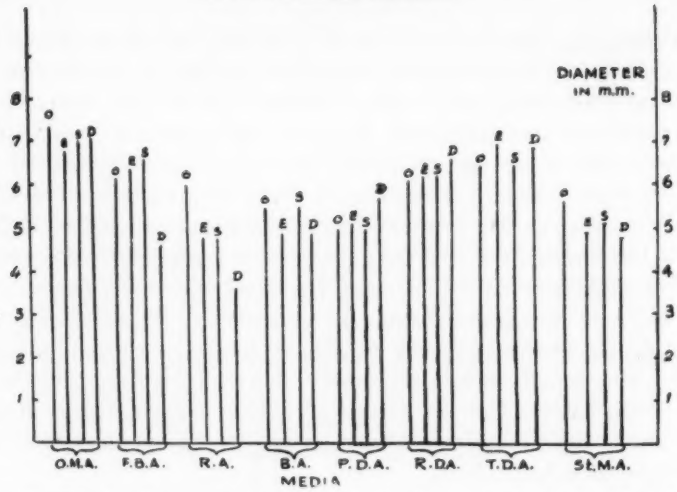
It is thus evident that morphological characters of the isolates do not afford much basis for their classification as they do not show significant differences. Nisikado (1917), though he has obtained similar results, has separated the strains on *Setaria italica* Beauv. into a new species—*Piricularia*

Setariæ Nisikado, basing his classification on the size of the individual cells of the spores, the basal appendage and the size of the germinating hyphæ. But the variations obtained by him within the groups, *viz.*, *Oryza* form and *Setaria* form, are too great and, therefore, any generalisation to include a particular spore in one group or the other may appear empirical and not rigidly scientific. Taking for instance the measurements of the conidia they vary from 14 to 40 μ × 6 to 13 μ in the *Oryza* form and 14 to 35 μ × 5 to 12 μ in the *Setaria* form. In the case of the individual cells of the conidium, taking the middle cell, it varies from 4·8 to 12·0 μ in the *Oryza* form and 4·8 to 8·3 μ in the *Setaria* form. It will now be difficult to say whether spores measuring say between 14 and 35 μ in length or 5 and 12 μ in breadth or having middle cells measuring between 4·8 to 8·3 μ belong to the *Setaria* or the *Oryza* form. The importance of taking morphological evidence in collaboration with physiological needs, therefore, no emphasis and this will be discussed at a later stage.

Physiological Studies

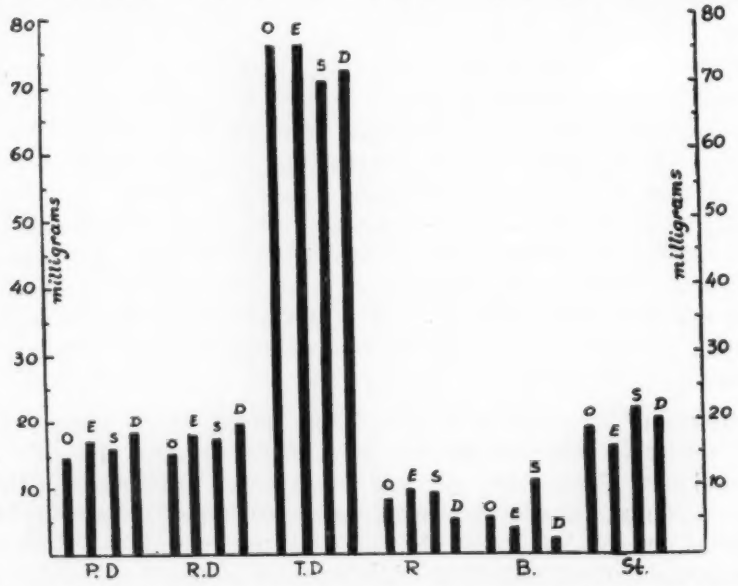
Materials and Methods.—The cultures of the four isolates used for the morphological studies were used for the physiological studies as well. Throughout the investigation standard mycological technique was followed and all the experiments were conducted at a constant temperature of 30° C. unless otherwise specified. For all the experiments a synthetic medium (Ramakrishnan, 1941) of the composition, glucose 10 gm., peptone 6 gm., K_2HPO_4 1·75 gm., $MgSO_4$ 0·75 gm., water 1 litre, agar 2% (for all solid media) was used unless otherwise stated. This is referred to as the Standard medium. In the case of growth studies care was taken to transfer equal quantities of the inoculum to the media in dishes or flasks. For obtaining the dry weight of mat the isolates were grown for a definite period in 50 ml. of the medium in 100 ml. flasks, the mat filtered through a gooch crucible, washed and dried in a hot water oven, to constant weight. All pH determinations were made with the help of a Quinhydrone Electrometric pH indicator and colour determinations with Ridgeway's (1912) colour nomenclature.

Growth of the strains on media.—Linear growth of the colonies of the four isolates on standard medium agar, Richard's agar, Brown's agar, oat-meal agar, French been agar and the decoction agars made of the leaf material of rice, ' ragi ' and ' tenai ', was determined. The amount of mat produced by the isolates in the Standard medium, Richard's medium Brown's medium and decoctions of leaf material of rice, ' ragi ' and ' tenai ' was also determined. The results are represented diagrammatically below:



GRAPH I.—Showing the average daily growth in diametre of the colonies of the four isolates in millimetres on different agar media.

O.M.A. for oat meal agar; *F.B.A.* for French bean agar; *R.A.* for Richard's agar; *B.A.* for Brown's agar; *P.D.A.* for rice leaf decoction agar; *R.D.A.* for 'ragi' leaf decoction agar; *T.D.A.* for 'tenai' leaf decoction agar; *St.M.A.* for standard medium agar. *O, E, S* and *D* represent isolates from *O. sativa, E. coracana, S. italica* and *D. marginata.*



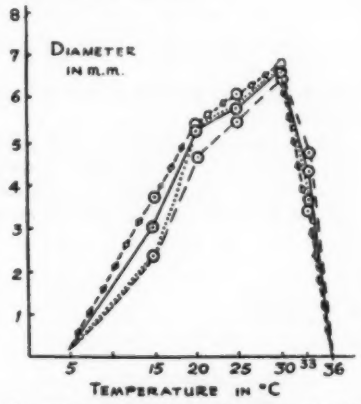
GRAPH II.—Showing the comparative amounts of mat produced by the isolates in different media.

(*P.D., R.D., T.D.,* indicate leaf decoctions of rice, 'ragi' and 'tenai'. *R., B.,* and *St.* indicate Richard's, Brown's, and Standard media.

O, E, S and *D* represent isolates from *O. sativa, E. coracana, S. italica* and *D. marginata.*)

The results of the above experiments agree with those of Nisikado (1927) in that the Madras isolates also produce good growth on the decoctions of their host material as did the Japanese isolates. The Madras isolates produced yellowish olive colouration in synthetic media containing sugars. While linear growth is best on 'ragi'-leaf decoction agar, the isolates produce maximum amount by weight of mat in 'tenai'-leaf decoction agar, showing that measurement of linear growth in terms of increase in diameter of the colonies does not always give a correct idea of the amount of mat produced.

Optimum temperature for growth.—Linear growth of the colonies of the isolates and the weight of mat produced by them at different temperatures are given in the following graphs.

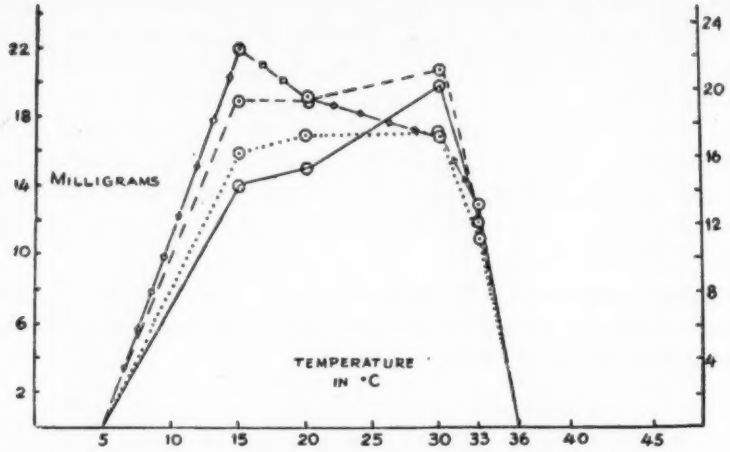


GRAPH III.—Showing the average growth in diameter of the colonies in millimetres at different temperatures.

———— isolate from *O. sativa.*
..... isolate from *E. coracana.*
— — — — — isolate from *S. italica.*
—△—△—△—△ isolate from *D. marginata.*

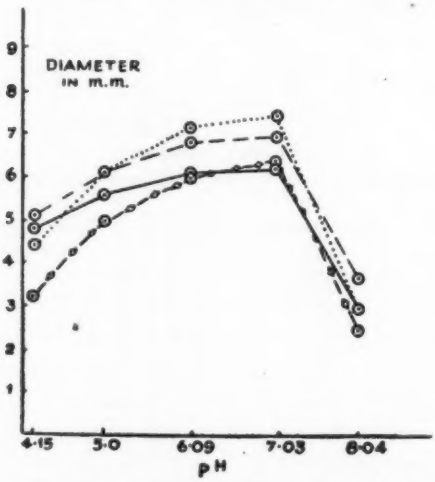
Thomas (1940) obtained best growth of a strain of *Piricularia*, isolated from 'ragi' at 29·5° C. Abe (1930) and Yoshii (1936) showed that 28° C. was the best for isolates from *O. sativa.* Nisikado (1927) has reported best growth of isolates from *Setaria* and ginger between 23 and 28° C., and the maximum temperature at which growth of the strains from rice was possible at 36 to 37° C.

Effect of pH of the medium on the growth of the isolates.—The following graphs show the rate of linear spread of the isolates and the weight of mat produced by them in the Standard medium at different pH levels.



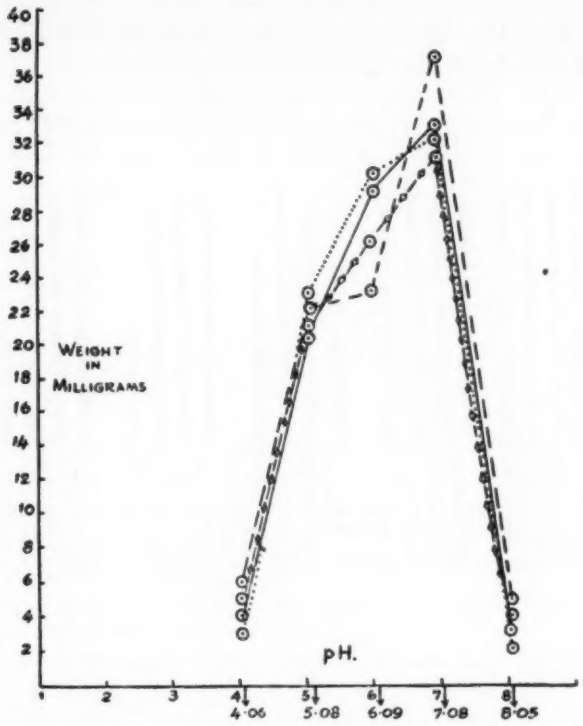
GRAPH IV.—Showing the comparative amounts of mat produced by the isolates in liquid medium at different temperatures.

——————·—— isolate from *O. sativa.*
..... isolate from *E. coracana.*
— — — — — isolate from *S. italica.*
—△—△—△—△— isolate from *D. marginata.*



GRAPH V.—Showing the average rate of daily growth of colonies of the isolates at different pH levels.

——————— isolate from *O. sativa.*
..... isolate from *E. coracana.*
— — — — — — isolate from *S. italica.*
—△—△—△—△ isolate from *D. marginata.*



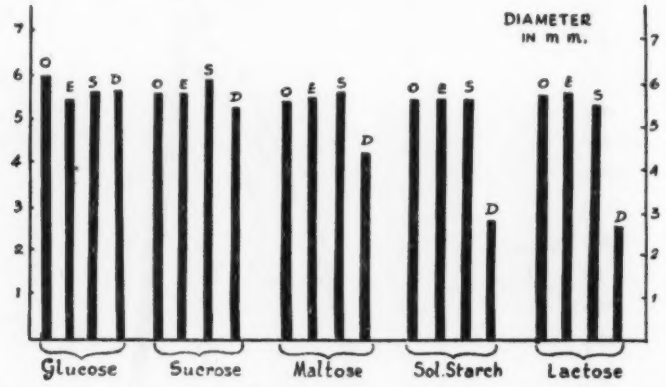
GRAPH VI.—Showing the comparative amounts of mat produced by the isolates at different pH levels.

———————— isolate from *O. sativa*.
..... isolate from *E. coracana*.
— — — — — — isolate from *S. italica*.
—△—△—△—△— isolate from *D. marginata*.

Nisikado (1927), with isolates from *O. sativa*, obtained best growth between pH values 5 and 10. A highly pathogenic strain grew best at pH 4·4. Thomas (1940) reports optimum growth of a strain from 'ragi' between pH 5 and 6. The present investigations show that round about pH 7 is the optimum for both dry weight and for radial spread irrespective of the isolates used. Though the variation in the weight of mat produced by the isolates is prominent the peak production of the mycelium both on dry weight basis and on radial spread was optimum for all the isolates at pH 7. During growth the isolates tend to bring the pH of the medium to round about 5 and 6.

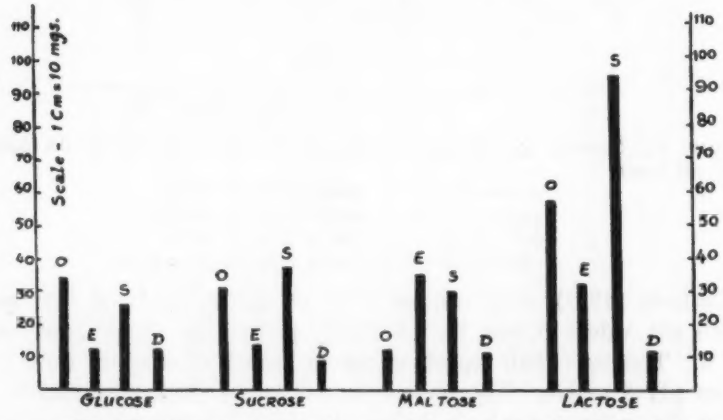
Influence of different sources of carbon in the medium on growth.—One of the following, *viz.*, glucose, maltose, lactose, sucrose, soluble starch and

cellulose was tried as the source of carbon in the medium at the 2% level. The results are shown below:



GRAPH VII.—Showing the average growth in diameter of the colonies of the isolates in standard medium to which different carbohydrates were added.

O, *E*, *S* and *D* represent isolates from *O. sativa*, *E. coracana*, *S. italica* and *D. marginata*.

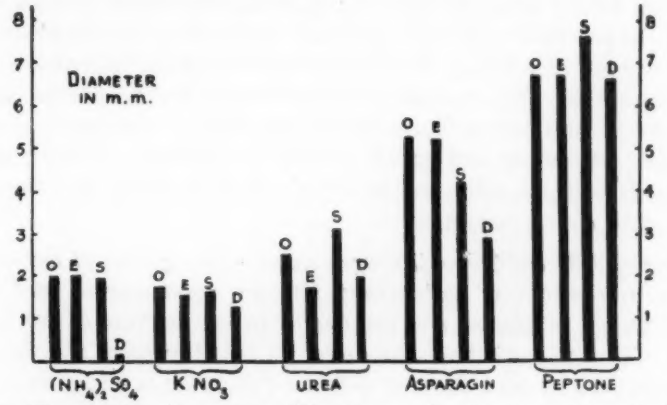


GRAPH VIII.—Showing the comparative amounts of mat produced by the isolates in media with different carbon sources.

O, *E*, *S* and *D* represent isolates from *O. sativa*, *E. coracana*, *S. italica* and *D. marginata*.

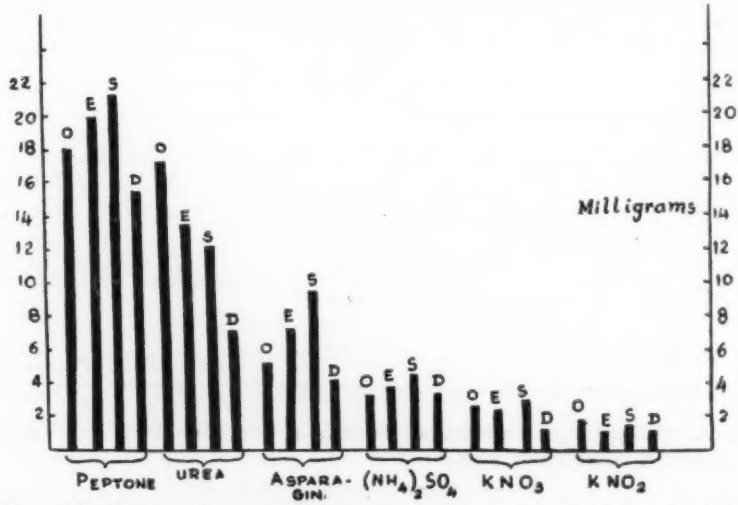
According to Tochinai and Nakano (1940) certain strains of *P. oryzæ* appear to be capable of utilising higher alcohols like glycerine and mannite as the carbon source. Among the carbohydrates tried here they prefer maltose, soluble starch and glucose, in the order of their mention. Yoshii (1936) reports pectin as a good source of carbon for growth of *P. oryzæ*.

Influence of different sources of nitrogen on growth.—The capacity of the isolates to utilise nitrogen from different sources was investigated using the following nitrogenous compounds in pure form: potassium nitrite, potassium nitrate, ammonium sulphate, urea, asparagin and peptone. Five grams of potassium nitrate was added to a litre of the standard medium.



GRAPH IX.—Showing the average daily increase in diameter of the colonies of the isolates in media with different nitrogen sources.

O, E, S and *D* represent isolates from *O. sativa, E. coracana, S. italica* and *D. marginata.*



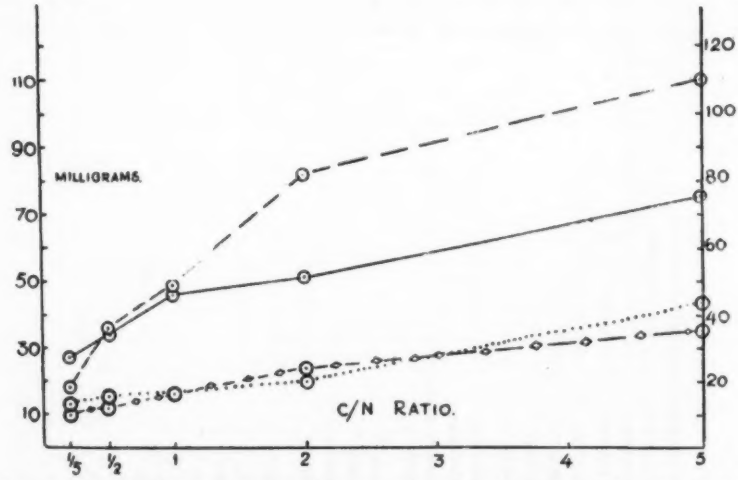
GRAPH X.—Showing the comparative amounts of mat produced by the isolates in media with different nitrogen sources.

O, E, S and *D* represent isolates from *O. sativa, E. coracana, S. italica* and *D. marginata.*

The other nitrogenous compounds were used in quantities calculated to contain an equivalent amount of nitrogen, *i.e.*, potassium nitrate 5 gm., potassium nitrate 4·25 gm., ammonium sulphate 3·1 gm., urea 1·5 gm., asparagin 3·3 gm., and peptone 5·9 gm. The above diagrams show the results.

Yoshii (1936) observed that *P. oryzæ* did not reduce nitrate and was found to be injured by nitrite. Tochinai and Nakano (1940) observed that the best source of nitrogen for *P. oryzæ* was peptone followed by sodium nitrate, asparagin, glutonic acid and acetamide. In the present investigation all the isolates do not favour potassium nitrite as the source of nitrogen in an agar substratum and growth is totally inhibited. Peptone gives the best growth both on solid and in liquid media showing that they prefer organic nitrogen to inorganic.

Effect of different Carbon/Nitrogen ratios on the growth of the isolates.— Different proportions of carbon and nitrogen sources were used keeping the total weight of glucose and peptone at 16 gm. per litre of the Standard medium. The experiment was conducted in liquid medium and the weights of mat produced by the isolates at different C/N ratios is given in the accompanying graph.



GRAPH XI.—Showing the comparative amounts of mat produced by the isolates in liquid medium at different C/N levels.

———— isolate from *O. sativa.*
..... isolate from *E. coracana.*
— — — — isolate from *S. italica.*
—△—△—△—△ isolate from *D. marginata.*

It is found that the weight of mat produced increases as the C/N ratio increases and best growth is obtained at C/N = 5/1. Ramakrishnan (1941) also obtained similar results with isolates of *Colletotrichum falcatum.*

Enzymes produced by the isolates during metabolism.—The production by the isolates of the enzymes diastase, inulase, lipase, erepsin, amidase and trypsin was tested quantitatively by the method adopted by Uppal and Kulkarni (1937) in their work on *Fusarium*, based on the method of Crabill and Reed (1915). The production of maltase, sucrase, lactase and urease was tested *in vivo* by the method followed by Garren (1938) in his studies on *Polyporus abietinus.*

TABLE II

Table showing the production of the different enzymes by the isolates

Name of enzyme	Medium used	pH of medium	*Piricularia* isolated			
			Oryza sativa	*Eleusine coracana*	*Setaria italica*	*Digitaria marginata*
Diastase ..	Soluble starch	6·52	+	+	+	+
Inulase ..	Inulin	6·78	+	+	+	+
Lipase ..	Litmus cream agar	7·29	+	+	+	+
Erepsin ..	Casein agar	5·00	−	+	+	−
Amidase ..	Asparagin Rosalic acid agar	6·44	+	+	+	+
Trypsin ..	Egg albumen agar	6·35	+	+	+	+
Sucrase ..	Sucrose	6·52	+	+	+	+
Maltase ..	Maltose	5·93	+	+	+	+
Lactase ..	Lactose	6·26	+	+	+	+
Urease ..	Urea	8·80	−	−	−	−

+ shows production. − shows non-production.

It is seen from these results that all the four isolates produce diastase, inulase, lipase, amidase, trypsin, surcase, maltase and lactase and do not produce urease. The isolates from *E. coracana* and *S. italica* produce erepsin, while those from rice and *D. marginata* do not. Yoshii (1936) has detected oxidase and dehydrase in cultures of *P. oryzæ.*

CROSS INOCULATION STUDIES

Cultures of the isolates were maintained on sterilised leaf bits of the respective hosts. This facilitated a ready supply of spores whenever required as the fungi produced abundant conidia on these media and remained viable for long periods.

Seedlings were raised from healthy seeds in small pots. Garden soil was used for raising *S. italica* and *E. coracana* while soil from rice fields was used for raising rice and *Digitaria* seedlings. In each experiment 25 pots of

the seedlings were raised for each host inside glass cages which had been previously disinfected with a spray of 2 in 1,000 mercuric chloride solution, five pots in each cage. When the seedlings had grown to a height of about a foot, the seedlings were thinned out so that each pot contained about four healthy seedlings. The chambers were all kept in the shade in the pot-culture house. The experiments were conducted during the months November to January as infection by *Piricularia* was maximum in the fields during these months at Coimbatore.

Spore suspensions of the isolates were prepared in sterile atomisers. The inside of the cages was given a spray of clean water with a sprayer to raise the humidity, as high humidity favours infection by *Piricularia*. The spore suspensions of the isolates were sprayed on the plants in the four chambers each chamber receiving the spores of one of the isolates. The cages were kept closed. The plants in the fifth chamber formed the uninoculated controls.

Observations were made daily for the characteristic symptoms of infection by *Piricularia*. The symptoms, namely the leaf spots, appeared within 4 to 7 days after inoculation. The experiments were repeated thrice over two seasons. The following hosts of *Piricularia* were taken up:

1. *Oryza sativa* Linn. .. rice strain G.E.B. 24 of the Paddy Specialist, Coimbatore.
2. „ .. Adt. 10.
3. *Eleusine coracana* Gaertn. 'ragi' strain E.C. 593 of the Millets Specialist, Coimbatore.
4. *Setaria italica* Beauv. .. 'tenai' local Coimbatore variety.
5. *Digitaria marginata* Link. .. seeds obtained from the Millets Specialist, Coimbatore.

Variety G.E.B. 24 of rice is a fairly resistant strain evolved by the Government Economic Botanist, Coimbatore. Adt. 10 is a very susceptible variety grown in the deltaic tracts of the Tanjore District of Madras.

The results are given below:

Piricularia isolated from	Hosts tried				
	Rice G.E.B. 24	Rice Adt. 10	*Eleusine coracana*	*Setaria italica*	*Digitaria marginata*
O. sativa ..	−	+	−	−	−
E. coracana ..	−	−	+	+	−
S. italica ..	−	−	+	+	−
D. marginata ..	−	+	+	−	+
Control ..	−	−	−	−	−

+ indicates infection. − indicates non-infection.

It may be mentioned here that the isolate from *E. coracana* when infecting *S. italica* forms circular spots on leaves while the isolate from *S. italica* forms spindle-shaped leaf spots on *E. coracana* when infecting it. This shows that the nature of the leaf spot is due to certain qualities of the leaves of the host and not of the pathogen.

It may be concluded from the results of the experiments that the isolate from rice is distinct in that it infects only rice. The isolates from 'ragi' and 'tenai' each infects the host of the other in addition to its own. The isolate from *Digitaria*, in addition to its own host, infects 'ragi' and rice. These observations agree with those of Thomas (1940), who reports that the strain from 'ragi' and 'tenai' both infected 'ragi' and tenai' but not rice. Nisikado (1927) was not also able to get infection on grasses and cereals other than rice with *Piricularia* isolated from rice.

Discussion and Conclusion

There has been endless controversy and difference of opinion whether characters relating to morphology alone should form the basis for classification of the fungi into groups or whether their physiology, pathogenicity and host relationship should also be taken into consideration. Where there is marked morphological difference between the given members of the genus, it is an easy matter. But, when morphological differences are not very marked or are absent, the question must be asked whether classification should be based on such hair-splitting differences as variation of one or two microns in spore and mycelial measurements.

Turner (1940) compared *Ophiobolus* sp. from oats with isolates of *O. graminis* Sacc. from wheat, and found it difficult to distinguish one group from the other with the help of physiological characters. On the other hand marked differences were noticed in the length of the ascospores of the isolates from wheat and the ones from oats. All the isolates, however, produced similar symptoms in susceptible hosts. In spite of the differences in the morphological characters and in the host relationship, Turner (1940) considered the isolates from oats only as a new variety of *O. graminis* Sacc., *viz.*, O. *graminis* Sacc. var. *Avenæ* Turner, because of their similarity in cultural behaviour and symptoms produced in the susceptible hosts.

Leonian (1932), working on the pathogenicity and viability of *Fusarium moniliforme* Sheldon, deplores the common notion that dissociants "played havoc with taxonomy while on the contrary they are of inestimable aid in bringing order out of chaos. We have been in the habit of describing the species according to its morphological characters; we have usually failed to make intensive cultural studies, and have endeavoured to formulate fundamental

truths by superficial observations. To many of us form and size of spores and reproductive bodies constitute the sum total of the mycological concept, and the vital life processes of the fungi have little taxonomic value in our scheme of classification."

Leonian (1925) in an earlier work employed certain physiological features as manifest on solid agars and the host relationship to overcome the uncertainty of a purely morphological classification of some Phytophthoras, for the specific distinction within the genus were very limited, as its members exhibited remarkable uniformity of morphological characters. He believes that the average of all the morphological, physiological and pathological features should form the specific sphere.

Padwick (1939), while criticising Wollenweber and Reinking (1935) for their " indiscriminate use " of morphological and physiological characters in their scheme of classification of the Fusaria, advocates the adherence to their system for, he says, " Inadequate though it may be, there is nothing better." He would base specific rank upon characters easily recognisable " under standard conditions available in all moderately equipped mycological laboratories ". According to him, classification based on the use of host parasite relationship must be given a rank lower than the species, for it demands the use of a pure line of host and definite conditions such as temperature and moisture as has been shown by workers at Wisconsin, and soil conditions as shown by Mundkur (1936) working with the cotton wilt in India.

In the light of the above and the results obtained during these studies, it might be considered how the four isolates studied could be grouped. Of the four isolates, the one on *Oryza sativa* Linn. has been given a specific name *P. oryzæ* Br. e Cav. ever since 1892. Nisikado (1917) working on isolates of *Piricularia* from *O. sativa*, *Setaria* spp., *Zingiber* spp. and some grasses reports that these forms " showed many differences among themselves morphologically and physiologically " and has, on this basis given the form of *Piricularia* on *Setaria* spp. the status of a species namely, *P. Setariæ* Nisikado. A reference to the data put forth by him by way of morphological differences would show that emphasis could not be laid on measurements of the kind envisaged. The variations of individual measurements within the groups, namely, *Oryza* form and *Setaria* form, are too great and, therefore, generalisation to include any particular spore in one group or the other, may appear empirical and not rigidly scientific. It does not, therefore, appear proper that the separation of the *Setaria* form should have been based on such data. The isolates from *Eleusine*

coracana Gaertn. and *Digitaria marginata* Link, have not been assigned their systematic status so far.

From the results of the present observations it may be said that a study of the morphological characters does not help in distinguishing between the four isolates, there being no characteristic difference in the mycelia, conidiophores, conidia and chlamydospores. The only differences observed are in the nature of infection—(only foliar infection in the case of *Digitaria* and *Setaria*; and foliar, nodal and earhead infection in *O. sativa* and *E. coracana*, and the nature of the leaf spot, more or less roundish in *S. italica* and spindle-shaped in others). Even the nature of the leaf spots depands only on the host, as the *Setaria* isolate, when infecting *E. coracana* forms spindle-shaped spots and the *Eleusine* isolates when infecting *S. italica* form roundish spots. From the results of the physiological studies, the *Digitaria* isolate may be said to be distinct from the other three.

(1) It forms slate grey growth on oat-agar, while the others give pale gull grey growth. Unlike the other isolates, it does not grow well on Brown's agar.

(2) The *Digitaria* isolate does not very much favour cellulose as the carbon source, while the other isolates grow well in agar medium with cellulose as the carbon source.

(3) The *Digitaria* isolate does not grow in agar media with ammonium sulphate as the nitrogen source, while the other isolates, though they do not very much favour ammonium sulphate, grow fairly well with ammonium sulphate in the agar medium.

(4) 30° C. is the optimum temperature for growth, in liquid cultures of the isolates except the one from *Digitaria* which shows optimum growth at 15° C.

(5) Isolates from *E. coracana* and *S. italica* produce the enzyme erepsin, whereas the isolates from *O. sativa* and *D. marginata* do not.

It may thus be seen that the *Digitaria* isolate has certain physiological characters of its own.

It is very difficult to distinguish between the isolates from *O. sativa*, *S. italica* and *E. coracana* from their physiology, as they do not differ very much in their behaviour in culture media. The only differences are:

(1) The isolate from *O. sativa* differs from the other two by its inability to produce erepsin.

(2) The isolate from *E. coracana* shows better growth on agar medium with ammonium sulphate as the nitrogen source than with urea, whereas the others prefer urea to ammonium sulphate.

(3) In liquid medium maltose is the best source of carbon for the isolate from *E. coracana* while the other isolates grow best with lactose.

Cross inoculatfon experiments show that the *Piricularia* isolate from *O. sativa* is distinct from the other three isolates from *E. coracana, S. italica* and *D. marginata* in that it does not infect any host other than *O. sativa.* Nisikado (1927) also did not get infection with isolates from *O. sativa* on any host other than *O. sativa.*

The isolate from *S. italica* infects its host and *E. coracana* but not *D. marginata* or *O. sativa.* Similarly, the isolate from *E. coracana* infects in addition to its host *S. italica* and not *O. sativa* and *D. marginata.*

Nisikado (1917, 1927) did not study isolates from *E. coracana* and *D. marginata* nor did he use these hosts in his studies. He has doubted the ability of the isolates from *Setaria* spp. to infect *O. sativa.*

The isolate from *D. marginata* is able to infect *O. sativa* and *E. coracana* in addition to its host. It should be noted here that isolates from *O. sativa* and *E. coracana* do not infect *D. marginata.*

Summing up, the various *Piricularia* isolates can be classified into three groups:

(*a*) Isolates that attack only their natural hosts, *e.g.*, isolate from *O. sativa.*

(*b*) Isolates that attack their own hosts and one other, *e.g.*, isolates from *E. coracana* and *S. italica.*

(*c*) Isolates that attack their own hosts and two more, *e.g.*, isolate from *D. marginata.*

If morphology could alone be depended upon as the basis of classification it will be impossible to place the isolates systematically. If Leonian's (1925) suggestion of taking the sum-total of all the characters, morphological, physiological and pathological, is to be followed, it may be necessary to study more forms before arriving at some conclusion. The system of Wollenweber and Reinking (1935) of studying physiological characters in addition to morphological is somewhat similar to Leonian's (1925) suggestion and, indeed, has been emphasised by Padwick (1939) who has recommended the use of characters easily recognisable under standard conditions in all moderately equipped mycological laboratories and this presumably includes cultural studies as well. Padwick (1939) has also admitted of a classification based on the host relationship, though as inferior in rank to the species, if a pure line of the host and definite conditions of temperature and moisture

were assured. In the present studies, the hosts used were the susceptible varieties and the experiments were conducted under conditions most favourable for infection (under ideal weather conditions in Coimbatore during the months November to January, when the crops showed maximum infection in the field) thus incorporating the suggestions of all the workers in the experimental technique.

Nevertheless, it has been found difficult, if not impossible to draw bold lines of demarcation between subtle differences that exist among the different isolates of *Piricularia* studied here. Although very prominent and specific differences between the physiological behaviour and degrees of pathogenicity have been noticed among the isolates, it has been found impossible to detect significant morphological differences. This study, without making extravagant claims, has established the importance of attacking problems of classification of fungal parasites from three angles, namely, physiology, pathology and morphology, and the final analysis of the results to be undertaken by studying the three aspects interdependently and not independently. Further work with larger number of isolates from more hosts that the fungus *Piricularia* is known to attack, possibly grasses under the monocots and other hosts like *Theobroma cacao* Linn. among the dicots would enable significant comparisons easier and may result in establishing different species or varieties of the same species. Until such time, this work enables us to conclude that the various strains of *Piricularia* studied here can only be considered from a broad evolutionary point of view and they have to be provisionally placed as physiological races within the genus and species *Piricularia oryzæ*.

Summary

The morphology of four isolates of *Piricularia* from *Oryza sativa* Linn., *Eleusine coracana* Gaertn., *Setaria italica* Beauv., and *Digitaria marginata* Link., has been studied. No appreciable difference in the morphological characters of the isolates was noticed.

The physiology of the isolates was studied. A study of their growth on different media, the optimum temperature and pH range for their growth was made. The effect of different sources of carbon and nitrogen and their different ratios in the medium on the growth of the isolates was investigated. The enzymes produced by the isolates were qualitatively tested for.

Cross-inoculation studies were conducted under optimum conditions for infection of the hosts.

The studies reported in this paper have established the importance of attacking problems of classification of fungal parasites from three angles,

namely, morphology, physiology and pathology and these aspects considered interdependently and not independently. With the results obtained an attempt has been made to classify the isolates within the genus.

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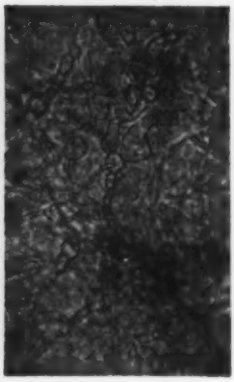

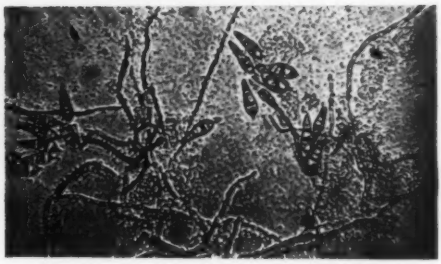

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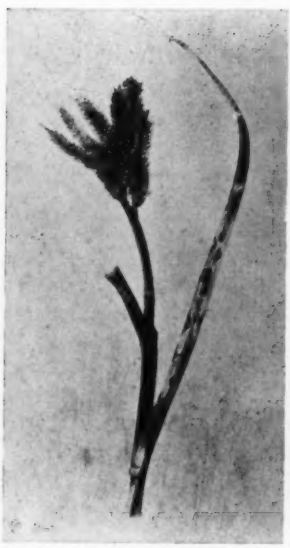

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Explanation of Plate

1. Photograph of two varieties of rice — one resistant and the other susceptible — seen side by side in an experimental plot at Coimbatore.
   - A. Susceptible variety completely destroyed by *Piricularia* attack.
   - B. Resistant variety unaffected.
2. Photomicrograph of mycelia from young cultures of *Piricularia* (mycelia straight and thin) × 360.
3. Photomicrograph of mycelia from older culture (mycelia variously contorted) × 360.
4. Photomicrograph of 3-celled top-shaped spores of *Piricularia oryzae* Br. e Cav. × 230.
5. Rice (*Oryza sativa* Linn.) affected by *Piricularia* (spindle-shaped leaf spots and darkened neck, node and grains) — diagrammatic.
6. 'Ragi' (*Eleusine coraceana* Gaertn.) affected by *Piricularia*. Note spindle shaped leaf spots with neighbouring spots coalescing. Also showing affected ear-heads — diagrammatic.
7. 'Tenai' (*Setaria italica* Beauv.) affected by *Piricularia* (Circular leaf spots, ear-heads not affected) — diagrammatic.

Note.—For photograph 1 the author is indebted to the Govt. Mycologist, Coimbatore and to Dr. T. S. Sadasivan.

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